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DOCTORAL THESIS

NEUROPHATIC PAIN

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To my family

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SUMMARY

Neuropathic pain (NP) embraces a broad range of conditions linked with a disease or lesion of the peripheral or central somatosensory system and its prevalence in the general population may be as high as 7-10%. Up to the present, the larger part of our understanding of pain mechanisms come from basic sciences studies which has resulted in a vast increase in our knowledge. The obvious issues in translational pain research reveal the limitations of certain experimental models, and on that account result of limitations in clinical research. In spite of such difficulties, the scientific community wish for a better understanding of pain mechanisms helped by the new insights of basic research. For that reason, the first of the three parts of this work aim to recognise additional changes in the somatosensory system through a series of experiments done in an experimental model of NP.

NP following peripheral nerve injury is associated with hyperexcitability in damaged myelinated sensory axons, which begins to normalise over time. We investigated the composition and distribution of shaker-type-potassium channels (Kv1 channels) within the nodal complex of myelinated axons following injury. At the neuroma that forms after damage, expression of Kv1.1 and 1.2 was markedly decreased. In contrast Kv1.4 and 1.6, which were hardly detectable in the naıve state, showed increased expression following injury. Within the dorsal root we noted a redistribution of Kv1-channels towards the paranode. Blockade of Kv1 channels with a-DTX after injury reinstated hyperexcitability of A-fibre axons and enhanced mechanosensitivity. Changes in the molecular composition and distribution of axonal Kv1 channels, therefore represents a protective mechanism to suppress the hyperexcitability of myelinated sensory axons that follows nerve injury.

To date, both basic and human data suggest that a lesion of afferent pathways is required for development of NP. In addition, numerous studies demonstrate that not one but various mechanisms can induce to NP. This not only evidence the complexity of NP, but also underline the clinical significance of identifying underlying pain mechanisms in every single patient. Because different management plans are required for different pain mechanisms, a mechanism-based treatment approach can guide clinicians to better outcomes. With these concepts in mind the second chapter of this study aim to clarify some links between one of the most novel techniques used in the diagnosis of NP, the Quantitative Sensory Testing (QST), which has been used to determine the pathological mechanisms functioning and the phenotype expressed. With the painDETECT Questionnaire as screening tool for NP, we analyse whether the overall PDQ score or its items reflect phenotypes of sensory loss in NP as determined by QST. The results of our study conclude that patients with loss of thermal sensation significantly more often reported pain evoked by light touch, and patients with loss of mechanical sensation

significantly more often reported numbness and significantly less often burning sensations and pain evoked by light touch. Although the PDQ was not designed to assess sensory loss, single items reflect thermal and/or mechanical sensory loss at group level, but because of substantial variability, the PDQ does not allow for individual allocation of patients into sensory profiles.

Finally, the last part of this thesis focuses its attention in the management of NP, more precisely in Complex Regional Pain Syndrome (CRPS) in children. This chapter review the evidence for invasive pain procedures along with presenting a management algorithm for paediatric CRPS, including invasive procedures for patients who do not respond to the conventional first-line treatment. We also report on the course and management of a series of children diagnosed with CRPS, applying the multidisciplinary management approach described above. We conclude that because of the severity and rapid progression of the symptoms in CRPS an early diagnosis of the condition together with comprehensive, and individualized multidisciplinary treatment offers children with CRPS the best opportunity for a complete recovery. Within this approach we encourage clinicians to consider invasive procedures as a reliable option of treatment.

RESUMEN

El Dolor Neuropático (DN) abarca, o acompaña, a una amplia variedad de patologías ligadas con enfermedad o lesión del sistema somatosensorial a nivel central o periférico, oscilando su prevalecía en la población general en torno al 7-10%. En la actualidad la mayor parte del conocimiento que tenemos de los mecanismos subyacentes al origen y desarrollo del DN han sido obtenidos de estudios en ciencias básicas. Los obvios problemas que surgen de la traslación en investigación médica revelan las importantes limitaciones de ciertos modelos experimentales, y desde esa línea se originan esenciales limitaciones para la investigación clínica. A pesar de estas dificultades, la comunidad científica escruta sin cesar nuevas y mejores ideas que nos ayuden a entender mejor la patofisiología del dolor basándose fundamentalmente en nuevos descubrimientos en el campo de las ciencias básicas. Por esta razón, la primera de las tres partes de las que se compone este trabajo tiene como objetivo el reconocimiento de cambios o hallazgos no descritos en el sistema somatosensorial en un modelo experimental de DN.

El DN tras un daño nervioso periférico esta asociado con la hiperexcitabilidad de los axones sensoriales mielinizados afectos, la cual comienza a normalizarse con el tiempo. Este trabajo investiga la composición y distribución de los canales de potasio voltaje dependientes de la familia Shaker (Kv1) en el complejo nodal de los axones mielinizados tras la lesión nerviosa. En el neuroma que se forma tras el daño, la expresión de Kv1.1 y Kv1.2 estaba considerablemente reducida. Por el contrario Kv1.4 y Kv1.6, que eran casi imperceptibles en el estado naive, mostraron un importante aumento en su expresión tras el daño. En la raíz dorsal se pudo demostrar una redistribución de los canales Kv1 hacia el paranodo. El bloqueo de los canales Kv1 con a-DTX tras la inducción de la lesión reinstauró la hipersensibilidad de las fibras A y aumentó la sensibilidad mecánica. Los cambios observados en la composición molecular y distribución axonal de los canales Kv1, representan por tanto un mecanismo protector que disminuye la hiperexcitabilidad de los axones sensoriales mielinizados tras un daño nervioso.

Actualmente, tanto la evidencia animal como la humana coinciden en que para el desarrollo del DN una lesión de las vías aferentes somatosensoriales es necesaria. Además, numerosos estudios demuestran que son varios los mecanismos que pueden inducir el desarrollo del DN. Esto no solo pone de manifiesto la complejidad del DN, sino que también destaca la importancia clínica de identificación de los mecanismos subyacentes del dolor en cada uno los individuos que lo sufren. Ya que diferentes planes de tratamiento son necesarios para el manejo de los diferentes mecanismos, un abordaje terapéutico basado mecanismos podrá guiar a los profesionales de la salud hacia mejores resultados. Con estos conceptos en mente el segundo apartado de esta tesis tiene como objetivo descubrir posibles asociaciones entre una de las mas novedosas técnicas utilizadas para el diagnostico del DN, el Test de

Cuantificación Sensorial (QST), el cual se puede aplicar para determinar los posibles mecanismos subyacentes activos, y el fenotipo sensorial expresado. Con el cuestionario painDETECT (PDQ) como herramienta de screening para DN, nosotros analizamos si el resultado obtenido para el PDQ o sus ítems reflejan fenotipos de perdida sensorial en DN demostrados con QST. Los resultados de nuestro estudio concluyen que los pacientes con perdida significativa de sensación térmica reportaban mas frecuentemente dolor evocado por tacto fino, y los pacientes con perdida significativa de sensibilidad mecánica reportaban mas frecuentemente adormecimiento, así como una menos frecuente sensación de quemazón y dolor evocado por tacto fino. A pesar de que el PDQ no fue diseñado para evaluar la perdida de sensibilidad, sus ítems individuales pueden reflejar la perdida de sensibilidad térmica y/o mecánica, pero debido a la importante variabilidad existente, el PDQ no nos permite la asignación de pacientes en perfiles sensoriales.

Finalmente, la ultima parte de este trabajo enfoca su atención en el tratamiento del DN, mas específicamente en el tratamiento del Síndrome de Dolor Regional Complejo (CRPS) en niños. Este capitulo revisa la evidencia científica actual para el tratamiento de este síndrome con procedimientos invasivos, además de presentar un algoritmo terapéutico para el manejo del CRPS pediátrico. Destacar que en este algoritmo se incluyen los procedimiento invasivos para aquellos sujetos no respondedores a los tratamientos convencionales de primera línea. Conjuntamente, este capitulo detalla el curso y manejo de una serie de casos de pacientes pediátricos con CRPS refractarios al tratamiento convencional y a los que se les aplicó de manera satisfactoria el algoritmo terapéutico descrito en este trabajo. La conclusión de este capitulo nos hace ver que debido a la rápida progresión y severidad de los síntomas en el CRPS solo con un diagnostico precoz acompañado de un manejo metódico, multidisciplinar e individualizado pueden ofrecer a estos pacientes la mejor oportunidad para una recuperación satisfactoria. Cabe destacar la inclusión de los procedimientos invasivos en el momento adecuado como una opción eficaz de tratamiento.

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INTRODUCTION

1. Chronic & Neuropathic Pain. An introduction to the problem

Chronic pain (ChP) is one of the most burdensome health issues facing the society today; its cost to Western countries is nearly the same of to the cost of cancer and diabetes mellitus combined (Vos et al. 2012). Reliable epidemiological research on ChP provides key information on prevalence and factors linked to its genesis and perpetuation. Enhancing our understanding of the details surrounding the disease will improve the clinical management, boundarying severity, and minimizing disability. There are well founded hypothesis that the recent estimations of global burden of disease have underestimated the contribution of ChP (Smith et al. 2007, Shmagel et al. 2016). The World Health Organisation (WHO) and other researchers forecast that for the 2030 the four leading responsible of global burden of disease will be coronary heart disease, road traffic accidents, depression and cerebrovascular disease (Vos et al. 2015). Unsurprisingly, ChP is a major co-morbidity linked to all of these; however, ChP is much more than a simple a co-morbidity of other injury or disease. ChP is to date acknowledged as a condition in its own right (Merskey 1994, Tracey and Bushnell 2009, Treede et al. 2015). Roughly one fifth of the adult European population suffer ChP (Breivik et al. 2006) and, in addition to the physical and emotional burden it brings, the monetary cost to society is vast, currently estimated greater than €200 billion per annum in Europe and $150 billion per annum in the USA (Tracey and Bushnell 2009).

Our understanding of the pathophysiology of ChP has increased substantially over the past 20 years, including from the periphery to the brain (Dubin and Patapoutian 2010, Apkarian et al. 2011, Baron et al. 2012). Nonetheless, we still do not understand why ChP develops in some people and not in others, yet we know that the magnitude or type of injury, psychosocial features, religious beliefs, occupation, education level, race or the postcode are not reliable predictors (Chou 2010). Extensive research into the genetics of ChP has also failed to predict the onset of the disease, possibly because the great number of genes involved and the contradictory research outcomes(Diatchenko et al. 2007, Møller and Jensen 2010). ChP treatment is usually a tedious and complex task, where the pain specialist and the patient should create a strong and genuine relation. It seems that despite great progress in multiple fields, little ground has been made (White et al. 2011, Rajapakse et al. 2014, Moulin et al. 2015).

Within the evolutionary picture the activation of any type of specialised nociceptor as the high threshold mechanoreceptors have a protective role, behaving as a warning system for threatening signals. However whereas inflammatory pain is adaptive, evolution has not succeeded to account for our increased capacity for surviving disease, trauma or iatrogenic aggressions intended to extend or improve quality of life. In such environments pain no longer is a helpful feature but becomes the disease itself. Nonetheless it is natural to think about pain as a homogeneous entity, this is unduly simplistic. In fact there are different types of pain, each with diverse pathophysiological and neurobiological mechanisms. The most recognised classification divides pain into two main types: nociceptive and neuropathic pain; however this classification is again over simplistic. Therefore, recently the new ICD category for "Chronic Pain" tried to include and divide the most common clinically relevant disorders. These disorders were separated into 7 groups: (1) chronic primary pain, (2) chronic cancer pain, (3) chronic posttraumatic and postsurgical pain, (4) chronic neuropathic pain, (5) chronic headache and orofacial pain, (6) chronic visceral pain, and (7)

chronic musculoskeletal pain. This division is important because it not only indicate the ethology and the neurobiological mechanisms, but also point treatment. Nociceptive pain can be categorised as visceral or somatic (Treede et al. 2015).

Neuropathic Pain (NP) is defined as pain resulting from injury to, or dysfunction of, the somatosensory system (Treede et al. 2008), thus tissue damage directly affects the nervous system, resulting in the generation of ectopic discharges that bypass transduction (Baron et al. 2010). NP is widely identified as one of the most complicated pain syndromes to manage, and outcomes are often disappointing. This is partly because the contribution of neuropathy to pain presenting in primary care may be unrecognised (Rodríguez et al. 2007, Leadley et al. 2012) and there is evidence of suboptimal drug use in the treatment of NP (Smith et al. 2007, Jongen et al. 2013, Helfert et al. 2014). Epidemiological research within the field of NP is problematic, and the reasons for this are diverse: the lack of a legitimate case definition that truly indicate the condition(s) under consideration, and that would be feasible to use in population-based studies; the heterogeneous quality of the studies, using inconsistent means of case ascertainment, and inclusion or exclusion criteria in which pain is not the major complaint (Torrance et al. 2006, Smith, Macfarlane, et al. 2007, Smith et al. 2012, van Hecke et al. 2013). Existing calculations of the general population prevalence therefore differ widely, and it is plausible that people experience neuropathic symptoms to a greater extent than have been diagnosed with a neuropathic pain-related condition (Torrance et al. 2006, Freynhagen and Baron 2009). Epidemiological NP studies are of great value in order to decide resource needs (educational, economical and clinical) in primary care and hospital settings, and to inform the prevention strategies and management targeting. As validated screening tools to identify NP have been developed (Torrance et al. 2006, Bennett et al. 2007, Haanpää et al. 2011, Mick et al. 2014), these have enabled questionnaire-based epidemiological studies, and there is a growing body of literature investigating the epidemiology of NP symptoms and conditions in the society. To date, the finest estimation of population prevalence of pain with neuropathic features is probable to lie between 7% and 10% (van Hecke et al. 2014).

NP is characterised by positive and negative symptoms embracing pain, hypoesthesia to touch, tingling, electric shocks and pins and needles (Woolf and Mannion 1999). Several nerve harmful stimuli at the central or peripheral nervous system can lead to NP, though the clinical features of the pain can be alike across the different neuropathic syndromes and aetiologies (Freeman et al. 2013). Although many forms of nociceptive pain, and some forms of neuropathic pain, may confer evolutionary benefits, chronic NP is at all times maladaptive. NP sufferers regularly manifest paradoxical sensory perceptions accompanied by pain as a paramount positive symptom combined with damaged induced downgraded sensations. These sensations are typically very unique and have not been felt previously by patients. This combination of signs of hyposensitivity and hypersensitivity is not unusual in neurological disorders; for instance, when spasticity appears after spinal cord injury or when parkinsonian tremor develops following degradation of the substantia nigra. Nevertheless, compared with these motor disruptions, pain as a subjective sensory symptom is difficult to measure and embrace not only physical features, but also psychological and emotional attributes (Gustin et al. 2015, Thacker 2015). The distinctive sensory abnormalities are key findings to diagnose and classify NP appropriately, and to differentiate this from other pain patterns. Major challenges in development of a targeted holistic approach to NP management cover appropriate diagnosis of the aetiology and mechanisms underneath, recognition of the type of pain and assessment of its pattern and its sensory profile, and determination of the right treatment.

2. Pathophysiology

NP is a highly complex clinical issue and has behaved as an enigma for pain researchers and doctors for a long time, being really complicated to identify and manage. To appreciate the complexity of NP, we should recognise the physiological transmission of the nociceptive signal. Noxious stimuli such as chemicals, inflammation, heat and pressure activate nociceptors, namely the prostaglandin receptors or acid-sensing ion channels (ASIC). This leads to influx of calcium and sodium ions, following in depolarisation of the cell. When the membrane depolarisation reach the threshold potential, this depolarisation is transmitted to the cell body, which is located in the dorsal root ganglion (DRG), and next carried up to the dorsal horn within the spinal cord. At this point, the neurones release neurotransmitters (mostly glutamate and substance P) into the synaptic cleft, which consequently depolarises the postsynaptic neurones and has both excitatory and inhibitory interneuronal reactions. Following, the depolarisation is transmitted to higher areas within the CNS by the ascending tracts, where it may be processed as the experience of pain. The higher centres may then respond by sending an inhibitory or excitatory signal back to the spinal cord by the descending pathways (Woolf and Ma 2007, D'mello and Dickenson 2008, Stein et al. 2009).

The scenario described above is very different to the one in NP, where numerous changes take place following damage to a sensory nerve. There can be peripheral disruptions generating abnormal impulse origin and transmission or/and central disturbances, causing abnormal processing of the nervous input.

2.1. Peripheral mechanisms

Once tissue damage occur, inflammation and reparatory processes arise, guiding to a hyperexcitable state recognised as peripheral sensitization. In the majority of people, this situation settles as healing occurs and inflammation reduces. But, when nociception perseveres because of repeated stimulation from ongoing disease or tissue damage, the alterations in primary afferent neurons may continue.

Numerous circumstances can play a part in peripheral sensitization. Inflammatory mediators like substance P or calcitonin gene related peptide (CGRP), which are released from the neurone, elevate vascular permeability, resulting in increasing the oedema and the release of other products with pro-inflamatory features such as prostaglandins, bradykinin, growth factors, and cytokines. These substances sensitise nociceptors, leading to lowered firing thresholds and contributing to ectopic discharges (Woolf and Ma 2007, Stein et al. 2009).

Ectopic discharges can lead to spontaneous pain and may arise from the DRG to any other sites along an injured nerve, or even from uninjured adjacent fibres (Chen and Devor 1998). The process by which adjacent uninjured nerve fibres become excited as a result of non-synaptic "cross talk" is known as ephaptic transmission (Devor and Wall 1990). Allodynia apply to pain originated by normally non-painful stimuli, and it usually ensues from decreased stimulation thresholds. Hyperalgesia apply to magnified pain perception as an outcome of injured peripheral nerve nociceptive fibres, and it can be divided as primary or secondary. Primary hyperalgesia results in damaged tissue as a consequence of sensitisation of peripheral nociceptors; while secondary hyperalgesia appears in proximate undamaged tissue due to sensitization within the CNS. To a certain degree, this can be originated by ephaptic transmission or the

enlargement of receptive fields of injured nerves, or both (Costigan et al. 2009).

Following nerve damage, numerous genes that influence nerve function are down-regulated or up-regulated, and this can have an important effect on membrane excitability, transmission and transduction. Since gene expression modifies cellular features, this may result in a nerve phenotype transformation, demonstrating changes such as the expression of neurotransmitters usually expressed by C fibres (such as substance P or CGRP) now expressed in other type of fibres (Navarro et al. 2007). The phenotype switch has been proposed as one of the main mechanism underlying the pathogenesis of NP, and more particularly of allodynia (Sandkühler 2009, Jensen & Finnerup 2014).

One of the most notable changes after nerve injury - and phenotype switching - is the unregulated expression of sodium channels around the terminal injury site of injured axons and within the DRG (Wang et al. 2011). During the last two decades preclinical studies have revealed that several types of sodium channels play an important role in pain. Following nerve damage, the expression of various of these channels decrease, the expression of others turns up de novo, and some others channels change their location at other cellular compartments. The rapid increase of heterotopic sodium channels, like Nav1.3, Nav1.7, and Nav1.8, can reduce the stimulation threshold and give rise to ectopic discharges, ending in spontaneous pain (Lai et al. 2003, Levinson et al. 2012). Additionally, the proliferation of sodium channels may precipitate central sensitization (Basbaum et al. 2009, Jensen and Finnerup 2014).

Certain types of potassium and calcium channels also play an important part in NP. After nerve damage, the expression of α2δ calcium channels is upregulated in and around the DRG, increasing excitability (Takahashi et al. 2010, Nieto-Rostro et al. 2014). These voltage gated calcium channels are the primary site of action for gabapentinoids, a first-line treatment for NP, which have been shown in preclinical studies to reduce hyperalgesia and spontaneous pain (Perret and Luo 2009, Taylor 2009, Pexton et al. 2011).

Within the physiological neuronal activation potassium channels facilitate a rapid transmembrane potassium efflux that affect action potential threshold, frequency and waveform. Since potassium channel opening repolarizes (or hyperpolarizes) the cell membrane, this property can restrict action potential generation and firing rate. Depending on the biophysical features and specific subcellular site in sensory neurons, potassium channel conduction is proposed to inhibit peripheral excitability by counteracting action potential generation at peripheral nerve terminals, lessen conduction accuracy across the axon, or bounding neurotransmitter release at central terminals. Besides, despite physiological sensory transduction does not depend on cell soma spiking, in some ChP conditions potassium channels can act as a brake to the spontaneous activity developing within the DRG or other ectopic loci as the neuroma (Rasband et al. 2001, Ocaña et al. 2004). After nerve injury a dramatic decrease in potassium conductance of peripheral nerve has been shown, correlating with the rising of hyperexcitability and pain behaviours in animal studies (Costigan et al. 2010, Tsantoulas and Mcmahon 2014). In the periphery, Kv1.1 and Kv1.2 are mainly detected in the soma and juxtaparanodes of medium-large DRG neurones, and are to a great extent reduced after axotomy; this may play a crucial role in the novel phenotype of hyperexcitability (Kim et al. 2002, Park et al. 2003, Costigan et al. 2010). Indeed, Kv1.1 loss-of-function outcomes in reduced firing thresholds, diminished heat and mechanical pain, while produce an increase of sensitivity thresholds (Chi and Nicol 2007). Conversely, decreased Kv1.2 activity is a factor in cold and mechanical NP by depolarising the resting membrane potential, diminishing threshold current, and boosting firing rates in myelinated neurones. In the CNS, potassium channel opening may contribute to increase nociception when, for example, the damaged neurone is part of an inhibitory circuit. Nonetheless,

the scientific evidence so far suggest that a collection of antinociceptive drugs act, or moderate their action, by opening potassium channels located at the CNS, brain or spinal cord (Hehn et al. 2012, Tsantoulas and Mcmahon 2014, Tsantoulas 2015).

Nerve injury also leads to upregulation of several receptor proteins like the transient receptor potential V1 (TRPV1). TRPV1 is placed on specific peripheral nociceptive endings and is physiologically activated by noxious heat at about 41°C (Christoph et al. 2006). Following nerve damage, TRPV1 is downregulated on damaged fibres but upregulated on undamaged unmyelinated fibres. The adjusted situation of TRPV1 and further sensitisation to heat stimuli by intracellular signalling may lead to spontaneous nerve activity generated by normal body temperature, if the threshold of TRPV1 is reduced to below 38°C (Fischer and Reeh 2007, Biggs et al. 2008). Clinically, people with the described pathophysiology can be identified by the presence of heat hyperalgesia in addition to ongoing burning pain. Likewise, abnormal responses to cold and topical application of menthol in these patients indicate that a nerve lesion triggered irregular function or expression of TRPM8, a cold-sensitive receptor of the TRP family(Staaf et al. 2009, Kambiz et al. 2014).

After nerve damage atrophic changes may cause a reduction in the size of the axon diameter and cell body, and eventually neuronal death (Wallerian degeneration) (Dubový 2011). Such changes may lead to a drop in intraepidermal nerve fibre density. Depending on the severity of the damage, this may result in negative signs and symptoms as loss of sensation or, paradoxically, increased pain and hypersensitivity, deafferentation pain (Costigan et al. 2009, Haanpää et al. 2011). Breaking the connection between a nerve and its end organ also disposes the nerve of nerve growth factor and other important substances and signals, which are crucial for the development and stability of the nerve (Dubový 2011). Nonetheless, electrodiagnostic studies may be normal in patients with a reduce intradermal nerve counting, where in response to local release of nerve growth factor, collateral sprouting may follow neuronal loss (Navarro et al. 2007).

Also within the periphery we should include the importance of the autonomic nervous system for the maintenance or enhancement of the pain by an abnormality in the sympathetic nervous system. Functional coupling between the sympathetic nervous system and somatosensory nerves after nerve damage dates back to 1864, when Silas Weir Mitchell reported his impressions during the American Civil War. Although the concept of sympathetically maintained pain usually associated to complex regional pain syndrome (CRPS), the same principles can be identified in other pain disorders, such as postherpetic neuralgia (Johnson and Rice 2014). The complex relationship between the somatosensory and autonomic systems most likely includes sympathetic sprouting into dorsal root ganglia, the expression of α-adrenoceptors on primary afferent sensory fibres, and decrease oxygenation and signalling in response to sympathetically mediated vasoconstriction (Nickel et al. 2012). Clinically, this situation can be expressed as colour and temperature changes in an affected body area, swelling, tissue atrophy, and pain worsened by cold weather or stress, which enhances sympathetic outflow (Gierthmühlen et al. 2014).

2.2. Central Mechanisms

Neuropathic pain modulates and modifies the neuronal spinal networks under pathological conditions, such as peripheral nerve injury and peripheral tissue inflammation (Todd 2010, Ellis and Bennett 2013). Some significant spinal components of NP mechanisms are synaptic plasticity in the form of temporal and spatial summation, augmented neuronal excitability of ascending nociceptive pathways that send pain signals to supra-spinal sites, and expansion of receptive fields of nociceptors and second order neurons (Dougherty and Willis 1992, Price 2000, Willis 2001). To a major extent these neuroplastic changes occur along nociceptive pathways in the spinal cord and in several brain areas, where nociceptive signals resulting by nerve damage are modulated by supra-spinal descending facilitation or inhibition (or both) that converges onto dorsal horn neurones. One consequence of this modulation is that the relationship between stimulus and response to pain is not always straightforward. The response of output neurones may be hugely modified via the interaction of numerous neurotransmitter systems within the spinal cord, all of which can be subject to plasticity and changes, especially during pathological conditions (Wu et al. 2010).

Accumulating evidence from various animal models of NP propose that NP might involve aberrant excitability in the dorsal horn, consequence of multiple functional changes following nerve damage (Woolf and Salter 2000). In addition, numerous studies have demonstrate that hyperexcitability induced by peripheral nerve injury might not be a result merely of changes in neurons, but rather of multiple alterations in glial cells, such as microglia, the immune cells of the CNS (Calvo and Bennett 2011, Calvo et al. 2012). In the dorsal horn glutamate is released from sensory afferents after an acute and more persistent noxious stimuli activates it, and it is fast AMPA receptor activation that is necessary for setting the initial baseline reaction of spinal dorsal horn cells to both tactile and noxious stimuli. Besides, if a repetitive and high-frequency stimulation of C-fibres happen, there is then a boost and prolongation of the response of spinal dorsal horn neurones to future inputs, so-called wind-up. In this circumstances it is likely that the co-release of peptidergic neurotransmitters, like substance P and CGRP, which are found in C-fibres together with glutamate, is behind the prolonged slow depolarization of the neurone and following removal of the NMDA block, thus allowing wind-up to happen. This augmented activity results from the activation of the NMDA receptor, which has been clearly demonstrate to play an important role in the hyperalgesia and enhancement of pain signalling seen in NP. The main mechanism by which the NMDA receptor acts is through the large influx of calcium ions happening when the channel is activated. Once inside the cell, calcium ions activate several effectors and encourage downstream cascades, such as nitric oxide synthase or ERK which can develop mechanisms of plasticity such as long-term potentiation (LTP). Similar plastic changes occur following acute high intensity C-fibre stimulation, peripheral nerve damage, and inflammation, and can conclude in the elevated responsiveness and activity of dorsal horn neurones. This phenomenon, known as central sensitization, is clinically present in NP sufferers as an increased response to painful stimuli (hyperalgesia), and pain arising from normally non-painful tactile stimuli (allodynia) (D'mello and Dickenson 2008, Costigan et al. 2009, Gao and Ji 2010). In addition, transmission at the level of the dorsal horn is under strong descending control from the brainstem, which can have facilitatory and inhibitory components. Accordingly, it seems clear that the final common pathological outcome of NP in the dorsal horn is enhanced excitatory transmission leading to pain; where the amplified excitability take place through a complex four-way communication between primary afferent terminals, dorsal horn neurons, microglial and astrocytes (and might also involve extra cell types like B-lymphocytes).

Immune cells of the CNS, microglia and astrocytes, contribute to the release of numerous growth factors, neuromodulators and inflammatory mediators. This, far from being a straightforward passive procedure triggered by degeneration of axon terminals; it is an active process started by injury signals released from damaged neurones. Scientific evidence is now clear when disclose that NP states can be linked with an intense inflammatory response which is not just a bystander phenomenon but, in some cases, lead the development and persistence of NP (Inoue and Tsuda 2009, Thacker et al. 2009, Calvo et al. 2012, Tsuda 2016). Glial cells can account for over 70% of the total cell population in the CNS, and are classified into astrocytes, microglia and oligodendrocytes. Microglia are known as resident macrophages within the CNS, and derive from primitive macrophages in the yolk sac (Ginhoux et al. 2010). During adulthood, microglia are present everywhere throughout the CNS and have small cell bodies showing branched and mobile processes, which might monitor the local environment in the CNS. Microglia react fast to a broad range of stimuli that threaten physiological homeostasis, including peripheral nerve injury. Numerous studies in the last decade have revealed how peripheral nerve injury lead to a substantial activation of microglia not only in the spinal dorsal horn, but also in supra spinal locations within the CNS (Inoue and Tsuda 2009, Ellis and Bennett 2013, Tsuda 2016). It has been showed that there is an astrocytic and microglial reaction within the rostral ventromedula (RVM) at the brainstem, which contributes to descending facilitation and enhanced pain related hypersensitivity after nerve injury (Banati 2002, Calvo et al. 2012). The disproportioned immune reaction is usually displayed among several models of NP including diabetic neuropathy, where activated microglia show dramatic changes in the expression of various genes, including cell-surface receptors for neurotransmission (e.g., purinergic receptors) and intracellular signaling molecules (e.g., mitogen-activated protein kinases [MAPKs]) and bioactive diffusible factors (e.g., pro-inflammatory cytokines and neurotrophic factors). Unfortunately, mechanisms underlying the spatial-specific activation of microglia either in the dorsal horn or in the brain remain to be determined (Milligan and Watkins 2009, Gao and Ji 2010). Importantly, molecular, pharmacological, and genetic manipulations of the function or expression of these microglial molecules have a large influence in pain behaviours and hyperexcitability of the dorsal horn pain pathway. Consequently, spinal microglia play a critical part in the pathologically enhanced pain processing in the dorsal horn, and microglial molecules might be potential targets for treating NP (Wen et al. 2011).

In addition to microglia, recent studies have also identified astrocyte-specific molecules, and have shown a critical role of spinal astrocytes in NP (Kawasaki et al. 2008, Tsuda et al. 2011, Xu et al. 2014). Under physiological conditions, astrocytes behave to 'mop up' molecules that are toxic or are in excessively high concentration, preserving extracellular homeostasis. In addition, astrocytes are important modulators of synaptic function (Kofuji and Newman 2004, De Leo et al. 2006). Astrocytic activation in animal models of pain take place various days following the damage or stimulus but is much longer lasting (Romero-Sandoval et al. 2008).

The output from the dorsal horn to higher centres in the brain is transmitted by spinal projection neurones along ascending pathways. A significant number of projection neurones is placed superficially in lamina I. It is estimated that approximately the 80% of such cells express the neurokinin 1 (NK1) receptor for substance P, a molecule largely released by nociceptive afferents, indicating that these neurones respond to noxious stimulation (Mantyh et al. 1997, Doyle and Hunt 1999). NK1-positive cells in lamina I have been shown to project to areas in the brain such as the periaqueductal grey (PAG) and thalamus (Baron et al. 2014, Wilcox et al. 2015), and into brainstem areas such as the RVM (Villanueva 2009, De Felice et al.

2011). In addition, a great number of projecting cells are also based deeper in the dorsal horn from lamina III-VI and these projects mainly to the thalamus, thereby making up a significant proportion of the spinothalamic tract. This ascending pathway transmits first and foremost sensory information, providing the sensory component of the pain experience. Descending pathways from higher centres as the brainstem are able to influence nociceptive signalling in the dorsal horn. These descending influences are both facilitatory and inhibitory in nature (Vanegas and Schaible 2004, D'mello and Dickenson 2008). Descending facilitatory pathways from the RVM in the brainstem have been proved to be implicated in the maintenance of pain following nerve damage (Vera-Portocarrero et al. 2006, Ossipov et al. 2010). Such excitatory influences encourage spinal central sensitization and assist nociceptive reflexes and their inappropriate tonic activation contributes to the pathophysiology of NP. The supraspinal descending facilitatory mechanisms have been proposed to play an important role in the a central mechanism by which the loss of sensory input resulting from the nerve damage is compensated (Dickenson et al. 2004).

Like descending facilitation, inhibitory pathways from the brainstem and other higher nucleus to the spinal cord may also undergo plastic changes in chronic NP. Various piece of research after peripheral inflammation shown several plastic modifications that can facilitate the inhibitory drive such as an increase in descending noradrenergic inhibition, coupled with an augmented efficacy of spinally administered a2-adrenoceptor agonists, as well as variations in other neurotransmitters such as serotonin or GABA (Green et al. 1998, Tsuruoka et al. 2003, Rahman et al. 2008, Dogrul et al. 2009). This enhanced inhibitory drive is presumably a homeostatic mechanism originated in an attempt to counteract an increased facilitatory drive and augmented spinal hyperexcitability (Fields and Basbaum 1979, Treede 2016). This dual control of the spinal cord by plastic circuits in the brain may be one important way in which the brain can modify pain processing, and could be the way by which anxiety, sleep, catastrophizing and coping can influence upon the level of pain perceived.

When the nociceptive signal reaches supra spinal structures it gets embraced in a complex multidimensional experience that do not only includes nocifensive and nociceptive but also cognitive and emotional-affective components. ChP is associated with functional and morphological changes in cortical and subcortical brain areas where to find objective markers for pain and related dysfunctions is unfeasible. NP as mentioned above should be understood as a progressive nervous system disease that results from poorly-defined neurophysiological and neurochemical changes. One critical challenge to understanding NP is determining to what degree the CNS, particularly the brain, contributes to NP's generation and maintenance. Neuroimaging has recently provided important clues to these challenges (Moisset and Bouhassira 2007). According to the current scientific evidence chronic NP may be a consequence of long-term plastic changes along the entire brain network associated to pain, formerly known "pain matrix" (Iannetti and Mouraux 2010). Besides peripheral nociceptors and the spinal cord, morphological and functional plastic changes also take occur in cortical and subcortical areas that participate in pain processing. Indeed, growing evidence now suggests that long-term plastic modifications in cortical networks may represent a possible basic mechanism underlying ChP, but unfortunately the neurobiological mechanisms of the various features of pain are only starting to emerge. A network of brain structures that process pain-related information has come to light due to numerous neuroimaging studies within the field (Apkarian 2004, Baliki et al. 2008, Tracey 2008, Apkarian et al. 2011, Lomakina et al. 2012). This complex network, former "pain matrix, uniformly incorporates primary (S1) and secondary (S2) somatosensory cortices, insular cortex, anterior cingulate cortex (ACC), and thalamic nuclei among other

less reported areas. Some of these locations have been associated with distinct features of pain, as S1 cortex is usually linked to sensory-discriminative aspects (Craig 2003), S2 associated with additional affective/ cognitive functions, or the insula and ACC are considered to be of great value for affective and cognitive aspects of pain (Ohara et al. 2005, Baliki et al. 2008, Sugimine et al. 2016). Yet scientific literature clearly shows how prefrontal cortical areas other than ACC and subcortical areas such as the amygdala have also an important place within the brain network for pain.

These brain areas may play a role in "secondary pain affect", which comprise the cognitive evaluation and the conscious awareness of pain (Wiech et al. 2008). Pain-related changes in these brain locations may be partly responsible for the emotional-affective and emotion-based cognitive consequences of pain. On the contrary, pain can be modulated by emotional - fear and anxiety- and cognitive -attention, expectation, or memory- factors (Baliki et al. 2006, Tracey and Mantyh 2007, Seminowicz et al. 2011). Unluckily, changes within the brain networks in NP are not always the same, activity changes in the thalamus and medial pain system are more homogeneous in spontaneous NP, whereas data on the involvement of cortical areas in allodynia appear more variable (Moisset and Bouhassira 2007). Non-identical presentations of allodynia may lead to different patterns of brain activity, indicating various pathophysiological mechanisms and suggesting that specific "allodynia networks" in the cortex may exist (Apkarian et al. 2005).

In addition to functional changes, morphological alterations at spinal and supraspinal levels have been reported in ChP. NP is associated with sprouting of nerve terminals in somatosensory cortex (Flor et al. 2006), apoptosis of spinal cord cells (de Novellis et al. 2004), grey matter density decrease in PFC associated with reduced cognitive abilities and thalamic atrophy (Apkarian 2004, Apkarian et al. 2011). Morphometric analysis demonstrated that chronic back pain specially in patients with a NP component is linked with approximately 10% of brain grey matter atrophy in prefrontal cortex and thalamus (Apkarian 2004). The reduction in grey matter was also detected in somatosensory cortex, temporal lobe and brain stem in addition to prefrontal cortex.

Nowadays, it remains to be found if decreased grey matter density is related exclusively or predominantly to a specific cell population (projection neurons, inhibitory interneurons, and microglia) or if different cell types are affected equally. In fact, numerous studies provide clear evidence of the maladaptive immune response is not just a bystander phenomenon within the brain but actively play an important role to persistent pain. The evidence for a part of the immune system in the origin and development of NP in patients is more circumstantial. Recruitment of immune cells and increased expression of pro-inflammatory cytokines in different areas of the brain has been shown in several studies (Ji et al. 2013, Loggia et al. 2015). However, the exact role of microglia and astrocytes in human pain disorders remains unknown.

In summary, neuropathic pain is a highly complex clinical situation developed after a series of maladaptive changes in neurones along the entire nociceptive pathway following nervous system damage. A substantial body of NP research points to various important contributory mechanisms including aberrant peripheral and central sensitisation, ectopic activity in nociceptive nerves, decreased inhibitory modulation, and pathological activation of microglia. The multitude of different mechanisms makes NP a highly complex condition, with a broad spectrum of presentations and notoriously refractory to treatment.

3. Clinical features

After peripheral tissue damage or inflammation, reversible modifications in the sensory nervous system result in the generation of pain hypersensitivity, a protective process that ensures appropriate healing of damaged tissue. Opposite, NP is originated after a real or potential damage to the nerve system itself and changes in its sensitivity may become persistent, leading to spontaneous pain, pain threshold may suffer a substantial drop such that innocuous stimuli lead to pain, and the amplitude and duration of its response to noxious stimuli are intensify. Because these changes in the somatosensory system in susceptible individuals can be irreversible, NP, once established, should be considered as an independent pathological condition of the nervous system in its own right (van Hecke et al. 2014). The majority of individuals do not develop NP after nerve damage and spite of the fact that only a few genetic polymorphisms have been reported that confer either an augmented susceptibility to development of NP, it is evident that genotype is an important contributor (Kehlet et al. 2006, Møller and Jensen 2010, Tremblay and Hamet 2010, Binder et al. 2011, van Hecke et al. 2015).

The first step in the clinical diagnosis of NP is to register the lesion or disease that is believed to be the source of it, and its anatomical location. Until not long ago, this was the entire diagnostic process, and frequently no effort was made to establish the actual neural mechanisms accountable for the development of the individual pain profile and how they may reflect in treatment options. A usual supposition is that a single aetiology origin NP in constant way. However, NP is highly heterogeneous, with multitude of presentation patterns revealing different combinations of genetic, environmental and aetiological factors, and specifically, the neurobiological processes they engage. Due to their mechanistic variety and dissimilar manifestations, these mechanisms originate a complex somatosensory profile that can be reflected as a constellation of negative and positive sensory signs and symptoms, a "pain fingerprint" (Scholz et al. 2009, Mahn et al. 2011, Freeman et al. 2013).

Commonly, negative symptoms are the earlier manifestation of damage to the somatosensory system and can be noticed by quantitative sensory testing in addition to clinical examination and to a more limited degree with the history or specific questionnaire. The origin of negative symptoms in peripheral neuropathies is direct damage to primary sensory neurones. This may lead cell death or altered transduction, conduction or transmission (or all of them) of sensory information. Loss of function can be showed across the whole sensory spectrum, being clinically proved by global numbness after a traumatic nerve injury for example, or it can affect specific modalities; for instance, we can associate an increased heat threshold due to deterioration of intraepithelial C-fibres is a common early manifestation of peripheral diabetic neuropathy or HIV neuropathy (Orstavik and Jørum 2010, Phillips et al. 2014).

A great number of patients with nerve damage only develop negative symptoms; some others also have disturbing positive manifestations due to aberrant plastic changes are engaged that boost pain sensitivity or guide spontaneous activation of the nociceptive pathway. Peripheral sensitisation typically take place after peripheral inflammation and involves a threshold drop and an enhancement in the excitability of the peripheral endings of nociceptors in response to sensitizing inflammatory mediators. This lead to stimuli that usually are felt as uncomfortable or slightly painful, such as a pinprick, becoming extremely painful (hyperalgesia) in the primary area of inflammation, and innocuous stimuli at the site of inflammation, such as light touch, warm or cool temperatures, being perceived as painful (Jensen and Finnerup 2014). The

presence of hyperalgesia and allodynia are debilitating consequences of peripheral nerve injury, and they can, potentially at least, arise as a result of spontaneous and aberrant activity raised anywhere along the nociceptive pathway. However, spontaneous sensations following peripheral nerve damage usually emerged as a result of hyperexcitability in the primary sensory neurone, resulting in ectopic abnormal activity not only at the injury site, but also at the soma or anywhere along the axon. Ectopic activity is a capital constituent of the spontaneous sensations that manifest following nerve damage originating paraesthesia, dysthesia, and pain. The sensations may be superficial or deep, continuous or episodic, and often has shock-like bursts and a burning quality, all of which may indicate engagement of ectopic activity in several type of fibres with distinct patterns of firing, in addition to subsequent central neuroplastic changes (Latremoliere and Woolf 2009, Sandkühler 2009). Simultaneously to the changes occurring at the damaged fibres, uninjured neighbouring fibres can potentially generate afferent input and consequently cause painful sensations (Wu et al. 2002); actually, it is postulated that this mechanism is an important source of neuropathic ectopic activity (Hehn et al. 2012, Jensen and finnerup 2014).

The discovery of central sensitization, a form of long-lasting synaptic plasticity in the dorsal horn triggered by nociceptors that facilitates nociceptive processing (Woolf 1983), led to the realization that amplification of incoming signals within the CNS has a very substantial role in the generation of clinical pain hypersensitivity in NP. In fact, central sensitisation account for a closer understanding of how low threshold A or C fibres can origin pain sensations, why repeated stimuli at a fixed intensity can lead to a progressive increase in pain, why there is a spread of sensitivity beyond areas of tissue injury or outside a damaged nerve territory, and why pain may long outlast a peripheral stimulus (Latremoliere and Woolf 2009, Woolf 2011, Hehn et al. 2012). Therefore, central sensitisation accounts for a mechanism that recruits naturally subthreshold synaptic input into a novel output from nociceptive neurones, lessen their threshold, increasing receptive field size, and altering firing temporal dynamics. All these changes give rise to the generation of dynamic mechanical allodynia in response to low threshold mechanoreceptor activation, pinprick or mechanical hyperalgesia and temporal summation.

4. Diagnosis

In the setting of clinical care for a patient suspected of having NP, careful history and physical examination and special laboratory tests serve physicians to formulate a differential diagnosis of the current condition; to allow better decisions making in management choice; and to follow-up individual responses to management (Haanpää et al. 2009, 2011). Diagnosis of NP is primarily found on history and physical examination even though other particular assessments are usually quite helpful. Clinical assessment should focus on excluding treatable conditions (for instance spinal cord compression or tumours), asserting the diagnosis of NP, and identifying clinical features that could contribute individualise therapy. Alike in other ChP conditions, assessment of a plausible neuropathic condition should include an evaluation of pain intensity, quality, location, temporal variation, functional impact on mood, sleep, and other activities of daily living and responses to previously attempted therapies (Gilron et al. 2013).

Accumulating evidence over the two decades has associated a collection of sensory signs and symptoms that are expected to be linked to NP conditions versus other non neuropathic conditions (Woolf and Mannion 1999, Bennett et al. 2006, 2007). Much of this information has emerged from the development and released of different NP screening tools including the Neuropathic Pain Scale (Feldman et al. 1994), the Neuropathic Pain Questionnaire (Krause and Backonja 2003), the pain DETECT (Freynhagen et al. 2006), or the Short-Form McGill Pain Questionnaire (Dworkin et al. 2009). Despite notable variability exist, the sensory quality descriptors "burning" (or "hot"), "tingling" (or " pins and needles " or " prickling), and "shooting" (or “electrical shocks") are included in all these tools, and these 3 descriptors are very likely the most distinctive of NP. Additionally it is clear that no single sign or symptom is pathognomonic of NP. The above, self-report, NP assessment tools can be of great help for the diagnostic characterisation of NP. Nevertheless, some of them have also been used to describe subgroups of NP patients in whom specific underlying mechanisms are operant. For instance, Baron et al. detect 5 different subgroups of sensory profiles evaluated using the pain DETECT questionnaire (Baron et al. 2009). In addition, pain quality descriptors can give more attributes in characterising therapy outcome beyond just global measures of pain intensity. By way of illustration secondary analyses of NP clinical studies show that drug therapy can specially lessen certain pain quality descriptors and have little effect on others (Jensen et al. 2009, Gilron et al. 2013, Bouhassira et al. 2014).

There is an important role of sensory testing in classifying a pain syndrome as neuropathic. The importance to the sensory evaluation is also reflected by the grading system for NP that has additionally been developed (Treede et al. 2008). It is based on four criteria: pain distribution (criterion 1), the link between pain distribution and the patient's history (criterion 2), confirmatory tests of neurologic status demonstrating positive or negative sensory signs enclosed to the innervation area of the damaged nervous structure (criterion 3), and further confirmatory diagnostic tests to identify the lesion or disease entity underlying the NP (criterion 4). Criteria 1 and 2 must be met to initiate the working hypothesis of plausible NP. Either criterion 3 or 4 must be met additionally to reach the grade of probable NP, while the grade of definite NP is achieved only when both criteria 3 and 4 are satisfied. Regarding those criteria, a reasonable QST result is, together with assessment of intraepidermal nerve fibre density and electrophysiological test procedures, regarded as a confirmatory diagnostic test to verify sensory signs in addition to sensory mapping (Haanpää et al. 2011).

4.1. Primary Sensory Assessment.

Reasonably basic bedside techniques can be applied to assess sensory abnormalities such as hyperalgesia to pinprick, blunt pressure, heat, or cold, hypoesthesia cold or touch, hypoalgesia or even allodynia (Haanpää et al. 2011, Freeman et al. 2013). Bedside sensory examination should include touch, pinprick, pressure, cold, heat, vibration and temporal summation. Responses can be graded as normal, decreased or increased to determine whether negative or positive sensory phenomena are involved. At present, it is generally agreed that assessment should be carried out in the area of maximal pain, using the contralateral area as control. Contralateral segmental changes following a unilateral nerve or root lesion cannot be excluded, however, so an examination at mirror sites might not necessarily represent a true control. Additional efforts should be made to understand how the identified sensory abnormalities account for a suspected neurological lesion. We must always have in mind that sensory nerve first assessed by clinical examination includes A-beta touch fibres (assessed for example with fingers, wisp of cotton, or soft brush), A-delta fibres (assessed with a metal straight pin or sharp wooden stick), and C fibres (assessed with a warm 40Cº object for example). To give an instance, postherpetic neuralgia or lumbar radiculopathy are quite focal conditions thus they are often associated with abnormalities along the affected dermatome, whereas other conditions such diabetic or HIV neuropathy are often related to distal and symmetrical sensory abnormalities (Baron and Binder 2004, Davies et al. 2006, Phillips et al. 2014).

4.2. Quantitative Sensory Testing (QST)

Patients with NP suffer from various sensory abnormalities that can develop in different combinations. It is thought that sensory signs and symptoms are closely linked to underlying mechanisms of pain generation, and it is therefore likely that precise analysis of the individual somatosensory pattern might facilitate a mechanism-based treatment strategy. Thus, it is important to assess the individual sensory phenotype as precisely as possible. Additionally to the self-report instruments and the basic bedside methods discussed above, the use of QST to assess sensory signs has manifested promising results in characterising underlying "mechanistic clusters" in NP as well as in predicting response to analgesic treatment with certain drugs. The term quantitative sensory testing refers to diagnostic procedures in which perceived stimulus intensities are referenced to test stimuli applied with defined intensities (Shy et al. 2003). Hence, QST is a "semi-objective" method, and therefore, a high grade of standardization is needed. A standardised QST protocol for routine use and clinical trials was launched by the German Research Network on Neuropathic Pain (Deutscher For-schungsverbund Neuropathischer Schmerz[DFNS]) in 2006 because standardisation is key to compare study results (Rolke et al. 2006). Sensory stimuli are applied to the skin or deep somatic anatomy to evoke a non-painful or painful sensation that can be quantified on a rating scale. QST uses a battery of mechanical and thermal stimuli (graded von Frey hairs, several pinprick stimuli, pressure algometry, quantitative thermal testing, etc) and assesses both positive signs (gain of function) and negative signs (loss of function) in the nociceptive and non-nociceptive afferent nervous systems. On the basis of QST, a novel classification and subgroupings of NP syndromes have been proposed. Similar to the way tumors are graded, patients are classified (LoGa classification) according to their function of small and large afferent fibres (Maier et al. 2008). Sensory phenotyping can also unravel subgroups of people with an anomalous endogenous pain modulation. The capacity to activate the descending pain modulatory pathways is individually variable and it can be quantified experimentally by measuring a reduction in pain perception with QST during simultaneous administration of a conditioning

painful stimulus at a distant body site (Yarnitsky et al. 2012).

If used properly, QST has started to play an important role in pain research. It has bridged a gap between basic researches, experimental studies using models of NP and pain patients, using the same test stimuli in all three contexts. This is contributing to understand the underlying mechanisms in NP syndromes (Arendt-Nielsen and Yarnitsky 2009, Chen et al. 2009, Cruz Almeida and Fillingim 2014). Additionally it is being a pivotal tool for transferring the mechanism-based treatment concept into clinical practice, where subgrouping sensory phenotypes, as an indicator of the pain processing pathway, is the basis to demonstrate that subgroups of patients with a distinct sensory phenotype certainly respond differently to a particular treatment. To give a recent instance of the advances in this area I can mention the work of Dumont et al. that published a clinical trial that explore in a randomized, double-blind, placebo controlled study the pain-relieving effect of oxcarbazepine in patients with surgical or traumatic nerve injury, polyneuropathy or postherpetic neuralgia. The investigators performed QST according to the LoGa classification in the beginning of the trial and stratified the individuals according to their sensory phenotype into 2 different groups: (1) "irritable nociceptor" with predominantly a "gain of function" and a preserved small-fibre nerve function and (2) "deafferentation type" dominated by sensory loss. This stratification stands on the hypothesis that ectopic activity from up-regulated sodium channels is primary responsible for hyperalgesia ("irritable nociceptor"), and therefore oxcarbazepine as a sodium channel blocker should have an effect in these patients. Despite oxcarbazepine is recommended as first-line therapy for trigeminal neuralgia, it plays a minor role in the treatment of other NP syndromes because of equivocal study results (Deng et al. 2016). This study evidence positive results and a treatment response depending on the sensory phenotype. For all patients, the number needed to treat (NNT) for 50% pain relief was 6.9. The NNT in the group with the "irritable nociceptor phenotype" was only 3.9, whereas the NNT was 13 for the "nonirritable nociceptor" phenotype (Demant et al. 2014).

4.3. Additional tests

Clinical neurophysiological techniques are crucial assessment tools in founding a diagnosis of NP with involvement of peripheral nerves. Nevertheless, clinical neurophysiology assess large fibres but is generally not useful in determining the possible involvement of small nerve fibres within NP. Various other techniques can be used in individuals with suspected small-fibre neuropathy. Aside from electrophysiological and clinical examination, other assessments comprise both structural tests such as structural imaging examinations of larger structures, skin biopsy analyses and sural nerve. The functional evaluations comprise, apart from QST, quantitative axon reflex measures, sudomotor function test, heart rate variability, contact heat-evoked potentials (CHEPs) and laser- evoked potentials (LEPs). An abnormal function of the sudomotor nerves with altered sweat has been identified as an initial neurophysiologic irregularity in neuropathies. There are several techniques to assess sudomotor functionality, being the most commonly used the quantitative sudomotor axon reflex test.

Furthermore, a novel devised objective "sweat test" has revealed the potential to identify and quantify early changes in sudomotor nerves due to partial denervation of single sweat glands (Provitera et al. 2010, Thaisetthawatkul et al. 2013). LEPs and contact heat-evoked potentials intend to evaluate noxious thermal information, transmitted by Ad fibres and C-fibres, employing in the case of the potentials contact heat stimulation, which stimulate a great number of nociceptors in the skin, whereas LEPs use high-energy

lasers to selectively activate individual nociceptors (Valeriani et al. 2012). Both methods are considered reliable techniques to study nociceptive pathways. Nevertheless, there is no definite correlation between LEPs and structural changes such as the intraepidermal nerve fibre density, indicating that loss of nerve fibres is not necessarily linked with loss of function of remaining nerve fibres (Truini et al. 2014). Additionally to these mentioned above, punch skin biopsy is a quick and minimally invasive technique with a high diagnostic yield and is at the present time a commonly used procedure in patients suspected of having small- fibre neuropathy (Ebenezer et al. 2007, Devigili et al. 2008, Malik 2014).

Over the past years, there has been some progress in obtaining additional quantitative measurements from the skin biopsies, including nerve fibre length densities, sweat gland innervation or axonal swellings. A strong point of skin biopsies is that they can be reproduced over time and allow the examiner to follow the progression of the disease quantitatively, and they can reveal changes in the small nerve fibres, which a standard neurological assessment is not capable of. An alike and entirely noninvasive technique is confocal microscopy of the cornea in which corneal small nerve fibres are quantified. This technique has been proved to be reproducible, sensitive, and very specific. Nevertheless, it only show loss of nerve fibres, leaving aside the ability to assess functionality of the remaining nerve fibres that can be intact, damaged, or overactive(Malik 2014, Petropoulos et al. 2014).

5. Treatment

NP management is still a great challenge because a large number of sufferers do not experience sufficient pain relief, as deduced from clinical trial outcomes and from clinical experience. This complexity in treatment might be consequence of the heterogeneity of NP pathophysiological mechanisms and the commonly coexisting emotional and psychological facets of ChP. Firstly, a rigorous diagnosis can disclose the source of pain; therefore, an appropriate management of it can result in partial or entire pain relief. When beginning symptomatic treatment, education of patients, embracing information on NP, the management plan, and feasible side-effects of any treatment -pharmacological or non pharmacological-, is indispensable to increase patient compliance. To avoid unrealistic expectations from NP sufferers on tolerability and efficacy, realistic management targets should be determined. Pain depletion of at least 25% is usually welcomed to be a clinically meaningful outcome (Attal 2011). Additionally to pain, both health-related quality of life and sleep disturbance should be assessed when evaluating analgesic efficacy (Smith et al. 2007, Gustorff et al. 2008). Furthermore, coexisting anxiety and depression might hamper successful pain management and should be recognised and targeted for specific treatment (Maletic and Raison 2009, Brod et al. 2014). In clinical settings, this complexity is taken into account by a multimodal therapeutic approach, involving pharmacological and non-pharmacological management options, such as physical therapy, psychological interventions such as cognitive behavioural, and occupational therapy (Kerns et al. 2010, Kozma et al. 2014, Casanova-García et al. 2015). In spite of the fact that the efficacy of such a multimodal biopsychosocial view has been routinely reported in ChP conditions other than NP, its strength in NP patients is well accepted (Garven et al. 2011). In those who develop phantom limb pain and CRPS, non-pharmacological treatments such as cognitive behavioural therapy and occupational therapy, as well as new methods such as graded motor imagery (including mirror therapy), have been shown to reduce pain and improve functionality (Katholi et al. 2014, Simons 2016). Likewise, invasive treatments have been reported as useful methods in neuropathic states when appropriately used (Slavin 2008, Pereira and Aziz 2014).

5.1. Mechanism-based therapy

ChP conditions are heterogeneous states with a large number of genetic, pathophysiological and psychosocial features, all of which are capriciously expressed in each person. As a result, current scientific and clinical approaches should study the different underlying elements as detailed as possible with the objective of tailor the management to the unique patient, a strategy which is called personalized pain medicine. NP was suggested in which pain syndromes are clustered based on the underlying pathophysiological mechanisms of pain causation rather than on the cause of the disease (Woolf et al. 1998). The idea supporting this was that it could be feasible and more appropriate to conduct specific treatments towards specific conditions looking at its particular nociceptive processing. Establishing a mechanism-based treatment.

Current understanding about pain mechanisms has grown exceptionally over the past years, becoming clear that numerous mechanisms both in the central or peripheral nervous system act alone or in combination in a single person. As a necessary condition for transferring the mechanism-based treatment idea into clinical practice, it is essential to develop diagnostic instruments that contribute determining pain mechanisms in patients and to cluster individuals accordingly. Until now, there is no biomarkers of pain

mechanisms, for that reason, clinicians have to rely on surrogate markers that are believed to be evenly matched to mechanisms of pain generation. A potential strategy to subgroup patients is to employ the profile of sensory symptoms and pain features, the so-called sensory phenotype, as a measure of abnormalities in the pain processing pathways. Both, animal and human scientific evidence support this approach in NP. It is clear now that nerve lesion triggers multitude of molecular and functional changes in every constituent of the nociceptive pathway. As a result, the nociceptive fibres develop irregular sensitivity and spontaneous activity. Presumably this changes in the patient nervous system lead to the perception of shooting pain sensations, spontaneous pain, in addition to thermal hyperalgesia. Following further somatosensory abnormalities, peripherally and central, can occur developing a unique and distinctive somatosensory profile for each individual.

In order to validate the mechanism-based treatment idea, it has to be proved that clusters of people with a specific sensory phenotype truly respond differently to a determined treatment. To date, the prospectively designed clinical trials that have been conducted using the method of sensory phenotyping as a stratification criterion and the retrospective response analyses using sensory phenotyping of NP patients at baseline of a trial are very promising (Herrmann et al. 2006, Baron et al. 2012, Yarnitsky et al. 2012, Kalliomäki et al. 2013, Martinez et al. 2013, Cruz Almeida and Fillingim 2014, Höper et al. 2014, Mainka et al. 2016). However, this technique should be implemented in following studies designs to eventually validate the mechanism-based treatment concept. Besides, simpler assessment techniques should be developed for reliably identifies subgroups of NP patients in the general practice.

5.2. Drug therapy for Neuropathic Pain

Over the last decade, several recommendations have been suggested for the pharmacotherapy of NP or certain NP conditions, especially post-herpetic neuralgia and painful diabetic neuropathies (Finnerup et al. 2015, Dubinsky et al. 2004, Dworkin et al. 2007, Attal et al. 2010, Bril et al. 2011). At the same time, novel pharmacological therapies have evolved and high-standard clinical trials have been completed. Besides, the risk of bias in collection, analysis and reporting data have been reduced due to the analysis of publication bias, together with the easier identification online of undisclosed or unpublished pharmacological studies. Different types of medications with analgesic effects have been shown to work better than placebo in clinical trials including patients with diverse NP conditions covering opioids, local anesthetic drugs, NMDA receptor antagonists, cannabinoids, antidepressants, anticonvulsants, botulinum toxin, topical capsaicin, and several other agents (Finnerup et al. 2015). Many of these drugs were initially developed for other indications, such as epilepsy or depression, and later on assessed in NP. Meta-analysis and systematic reviews of NP trials and development of management guides by several associations and societies have led to the current recommendations for gabapentin, pregabalin, tricyclic antidepressants, and serotonin-norepinephrine reuptake inhibitor (SNRI) antidepressants as first-line therapies; weak recommendations for lidocaine patches, high-concentration capsaicin patches, opioids, botulinum toxin A, and combinations of selected first-line agents; and weak recommendations against the use of cannabinoids and valproate (Finnerup et al. 2015). It seems clear then that the array of medications, and other treatment interventions, with demonstrated efficacy in NP is expanding. Therefore future research should not only define the finest use of existing drugs alone and in association, but should also determine medications that augment the magnitude of pain alleviation or the likelihood of a beneficial response.

5.3. Invasive Therapy for Neuropathic Pain

Patients with NP frequently do not respond sufficiently to the medication used alone or in combination with non-pharmacologic treatments and their pain is usually as a result termed refractory (Smith et al. 2012). Rather than continue endless pharmacological rotation that does not offer the wanted pain reduction or origin undesirable side effects, interventional treatments might be contemplated. Interventional pain management techniques embrace neural blockade, spinal cord stimulation, intrathecal medication, and neurosurgical interventions (Day 2008, Dworkin et al. 2013, Pereira and Aziz 2014). An up to date systematic review analysed the efficacy of various invasive techniques in several neuropathic pain conditions, such as post- herpetic neuralgia, spinal cord injury neuropathic pain and central poststroke pain, painful diabetic and other peripheral neuropathies, radiculopathy and failed back surgery syndrome, trigeminal neuralgia and trigeminal neuropathy and CRPS (Dworkin et al. 2013). This study concluded that evidence for the effectiveness of interventional techniques in NP is limited. It showed that just about half of the patients obtain lasting partial pain relief, an even a smaller number those whose relief was complete. However, it is important to highlight that careful evaluation of interventional and surgical pain options are fraught with numerous risks of bias associated to ethical and practical obstacle to treatment blinding and the employ of optimal sham, or other, control interventions; study patient dropouts attributable to intractability or severity and treated individuals; and the logistics and costs of follow-up and study duration. In addition, it must be remember that relative deficit of evidence of efficacy does not compulsory indicate evidence of lack of efficacy and therefore, rational interventional management of NP states should be contemplated an integral component of a more comprehensive approach that embraces pharmacologic and non-pharmacologic, non-interventional treatments.

5.4. The multimodal approach and non-pharmacological therapies

No single pharmacologic or interventional treatment abolishes symptoms of NP entirely. A complete management approach preferably involves the use of additional treatment strategies in an effort to ameliorate the situation. The objectives of any pain therapy comprise not only improve the symptoms but also recover physical function, reduce psychological distress, and enhance overall quality of life. It is necessary for clinicians then to clarify these goals to patients, set up appropriate expectations for pain relief, and engage individuals as active player in their management. Additionally, the physician should made comprehend the benefits of multidisciplinary management, making easier the active involvement in this multifaceted approach. It has been showed that people treated in multidisciplinary pain centres reduced pain intensity quicker, have reduced use of opioids, and enhance health-related quality of life compared with patients treated by general practitioners even when adhering to a pain management plan delineated by a pain specialist (Becker et al. 2000). Neurology, psychology, physical therapy, occupational therapy, and social support all contribute to the ultimate outcome of patients with ChP.

Attending the psychological component of chronic NP is crucial to success. Clinicians can support patients very easily, just by focusing on functionality and normal engagement in activities. Pain catastrophizing, is a well known predictor of poor response to pharmacotherapy and higher likelihood of treatment discontinuation; catastrophizing also predicts more extensive duration of pain, higher degree of disability, and poorer quality of life (Smeets et al. 2006, Toth et al. 2014). Cognitive behavioural therapy directly

addresses this maladaptive behaviour, aiding patients refine emotions and thoughts through education and training in coping skills and/or conscious confrontation of harming behaviours and thoughts (Turk 2003, Turk et al. 2010, Nijs et al. 2014). This kind of psychological approach has been demonstrated to provide benefit in NP patients not only reducing pain intensity but also providing a durable improvement in functionality, coping, pain related behavior, anxiety, and participation for patients suffering with chronic neuropathic spinal cord injury pain (Evans et al. 2003, Heutink et al. 2014). In addition any psychological intervention is being improved by the presence of an adequate social support. For that reason support group therapies have been embraced in pain management, easing patient engagement in the management strategy, allowing patients to share coping techniques and provide a supportive environment for positive reinforcement. Further psychoeducational wellness programs that augment awareness of social, intellectual, emotional, and spiritual factors are also effective in enhancing the overall quality of life and well-being of individuals with pain (Subramaniam et al. 1999, McGuire et al. 2015).

NP, as mentioned above, usually leads to reduce physical activity and functionality. Effective treatment must target both improvement in symptoms and functional restoration and mobilisation. A multidisciplinary approach to any NP condition is important; for instance in diabetic neuropathy recovering mobility and functionality is crucial for avoiding ulcers, contractures, and loss of sensation. In addition, exercise training in these patients improved perceived functional limitations, muscle strength and blood glucose regulation (Otterman et al. 2011). Other physical modalities such as transcutaneous electrical nerve stimulation or the use of osteopathic techniques may be applied to improve chronic NP (Kumar and Marshall 1997, Kuchera 2007, Arienti et al. 2011). Physical therapy for functional restoration is also commonly accepted as one the pillars in CRPS management (Zernikow et al. 2012).

6. Neuropathic Pain in Children

ChP affects approximately 6% of children and adolescents (van Dijk et al. 2006). The percentage of these children who develop NP is unknown. Some people suggest that the prevalence of ChP with neuropathic features in adulthood is similar to the one found in children (5%-10%) (Torrance et al. 2006, van Hecke et al. 2014). However, current evidence indicate that despite the fact that NP is seen in a remarkable part of referrals to paediatric ChP clinics the prevalence is much lower (Martin et al. 2010, Borsook 2012), and the conditions with which it is linked vary from those usually described in adulthood.

Accepted causes of NP during childhood embrace from situations where nerve damage has occurred, for instance, post-traumatic, phantom limb pain, postchemotherapy, and in some chronic states or infections such as HIV/ AIDS to genetic conditions affecting sensory nerve function such as Fabry ' s disease and erythromelalgia (Ramaswami 2008, Fischer and Waxman 2010, Walco et al. 2010). CRPS is an important idiopathic condition that occurs in both children and adults that is generally thought to be characterised by NP.

Thus evidence of NP has been detected in childhood, being neuropathy and sensory nerve dysfunction main features of most neuropathic conditions. However it is clear from our current knowledge of prevalence and prognosis that there are significant differences in the sensory response to nerve injury and CNS damages that appear to strongly relate to developmental age. By way of illustration, several clinical trials in animals have studied the effects of various types of nerve injury at different NP conditions and developmental ages, showing how mechanisms operating in childhood NP may vary from that in older age. Laboratory models of traumatic peripheral nerve injury have confirmed a diminished susceptibility to NP if the damage appear at a younger age; additionally this findings have been used to improve understanding of age-related changes in the pathophysiology of NP (Howard et al. 2005, Walker et al. 2009). Likewise, current literature suggest that symptoms of NP in childhood are generally less frequent and their severity relates to the underlying cause, age at onset or duration of disease (Vogel et al. 2002, Atherton et al. 2008).

Current recommendations for the assessment and diagnosis of NP are configured for adults, however they are usually extrapolated to children or adolescents. As there are no definite biomarker tests for NP the diagnosis is made on the basis of clinical indicators which is a well known issue of pain assessment in young children. Clinical history remains in childhood the centrepiece of diagnosis; nevertheless children may employ qualitative descriptors that are considered indicative of NP, such as shooting, radiating, burning, pricking, tingling, electricity-shock, stabbing, pins and needles, and pinching (Krane and Heller 1995, Wilkins et al. 1998, Walco et al. 2010). Obviously, numerous children may be unable to describe their pain using such terms but in any case pain history should contain the assessment of intensity, quality, temporal aspects of pain and response to treatment (Haanpää et al. 2011). Preferably all of these features -but most importantly pain intensity- should be evaluated using a validated scale; unfortunately, observational scales have mainly been designed for apply in adult acute pain settings and may not be reliable.

Like in adults physical assessment should aim to identify, confirm and locate the damage of the somatosensory system and report any associated neurological signs. Sensory abnormalities are more

complicated to acquire in children for many reasons such as communication or lack of previous data. For instance, reliable methods such as QST assess patterns of change in association with NP in adults, plus it is very important to establish a good communication between the patient and the investigator, both features that are usually diminish when assessing a child. Microneurography, functional brain imaging, electroneuromyography and skin biopsy may be indicated although again their use is mostly limited to research (Lebel et al. 2008). In addition, assessment of pain-related disability, sleep, quality of life, role functioning and mood should be usual assessments for children suffering of NP.

NP management at young ages is even more challenging and refractory than in adults. As mentioned above, chronic NP on rare occasions responds acceptably to a single analgesic medication or pain management strategy. Most pain states are highly modulated by activity in the CNS -brain and spinal cord-, which are in turn affected by cognitive and emotional factors and therefore a multimodal approach ground on a biopsychosocial pain model may always be engaged (Gatchel et al. 2007).Therefore, advances in NP management are likely to happen from a better understanding of the heterogenous mechanisms that are implicated at different stages of development, improvements in the accuracy of clinical diagnosis and a much more systematic and cautious documented approach to therapy. Correct management should embrace certain analgesic drugs ideally chosen on the basis of the pain mechanisms that are thought to be implicated, and a range of pain management strategies suitable to the clinical assessment of pain and any related functional impairment. Pharmacotherapy in NP is usually empirical and disappointing, as the particular underlying mechanism is rarely fully understood. Even so, a general approach based on an assessment of predictable benefits balanced against side effects is sensible.

7. Special Conditions. CRPS

Complex regional pain syndrome (CRPS) is a term coined by the International Association for the Study of Pain (IASP) to designate states characterised by spontaneous or stimulus-induced pain that is out of proportion to the inciting event and associated with a wide variety of motor and autonomic disruptions in highly variable combinations (Harden et al. 2007). The evidence points to CRPS etiology being a multifactorial disorder that is associated with an aberrant host response to tissue injury. Variation in susceptibility to perturbed regulation of any of the underlying biological pathways probably accounts for the clinical heterogeneity of CRPS (Marinus et al. 2011). There are many etiological pathophysiological affairs that have been accused in development of CRPS, however three major pathophysiological pathways have been identified: aberrant inflammatory mechanisms, vasomotor dysfunction, and maladaptive neuroplasticity. Additionally, it seems clear that inter-individual differences in the extent to which these mechanisms are affected account for the clinical heterogeneity of the disorder (Bruehl 2010). This syndrome steeped in uncertainty and commonly inaccuracy. There are no fully accepted guidelines that can be used to the diagnosis and would filled definitions of evidence-based medicine. Indeed, there are nearly as many diagnostic criteria as there are denominations to this condition. The umbrella term CRPS can be currently separated into type I and type II. CRPS I is meant to embrace the formerly known sympathetic dystrophy and similar disorders without a nerve injury; while CRPS II take place following damage to a peripheral nerve (Harden et al. 2007, Borchers and Gershwin 2014). To date the amount of trials that have included appropriate controls and have enough numbers of participants to permit statistical analysis with acceptable power calculations is minor. This has result in over-diagnosing and often use of excessive pharmacotherapy and even unneeded surgical interventions (Maihöfner et al. 2010).

In the last decade it has also become a well-established entity in children and adolescents although it counts with similar problems than it adults (Berde and Lebel 2005). CRPS remains probably under diagnosed and failure to distinguish CRPS leads to delayed management, unneeded diagnostic tests and inadequate treatment, which can worsen the situation and aggravate suffering. Early diagnosis, appropriate referral and treatment are essential in diminishing pain and improving function in children with CRPS (Stanton-Hicks 2010, Logan et al. 2013).

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REASONING FOR THIS STUDY AND HYPOTHESIS

Neuropathic pain embraces a broad range of conditions linked with a disease or lesion of the peripheral or central somatosensory system and its prevalence in the general population may be as high as 7-10%. The attraction in the pathophysiology of NP has augmented over the last decades with an exponential growth in the amount of clinical trials. Up to the present, the larger part of our understanding of pain mechanisms come from basic sciences studies which has resulted in a vast increase in our knowledge, these information require to be interpreted with carefulness due to the limitations associated with preclinical trials. The obvious issues in translational pain research reveal the limitations of certain experimental models, and on that account result of limitations in clinical research. In spite of such difficulties, the scientific community wish for a better understanding of pain mechanisms helped by the new insights of basic research. For that reason, the first part of this work aim to recognise additional changes in the somatosensory system through a series of experiments done in an experimental model of NP, which help us to acquire a better understanding of pain mechanisms.

To date, both basic and human data suggest that a lesion of afferent pathways is required for development of NP. In addition, numerous studies demonstrate that not one but various mechanisms can induce to NP. Significantly, a great number of these mechanisms are not based on the cause of the disease, the same mechanism can be detected in different conditions. In one single patient, several mechanisms can be implicated and diverse mechanisms could be the origin of the same symptom. This not only evidence the complexity of NP, but also underline the clinical significance of identifying underlying pain mechanisms in every single patient. Because different management plans are required for different pain mechanisms, a mechanism-based treatment approach can guide clinicians to better outcomes.

One of the main problems in the management of these syndromes is presumably the fact that treatments are apply in a uniform manner whatever the clinical picture, while these neuropathic states are in fact very heterogeneous. Important clinical progress has been made in this area in the last few years, following the validation of novel clinical tools and the standardisation of sensory profile paradigms enable improvements in the clinical characterisation of these conditions. Numerous studies have proved that NP is a consistent clinical entity, but it is multidimensional with regards to its clinical expression, with dissimilar sensory profiles, potentially indicating particular pathophysiological mechanisms. This new concept of NP should refine the characterization of the patient profiles in clinical trials and make available essential data for the development of new and more clinically sound translational approaches.

A route to advance at this stage in research clinical settings is to hypothesise that pain mechanisms can be deduced by studying individual symptoms and signs patterns with the above-mentioned methods. By studying the effect of treatment that targets these proposed mechanisms, the concept of mechanism-based treatment can be validated. This approach will allow design of clinical studies more focused on a mechanism-related symptoms and signs treatment approach instead of aetiology-based trials. So far, the current data contribute to understand the links between at least some clinical symptoms and suggested underlying mechanisms, however there is still a long pathway to walk in this field. The second part of this thesis aim to establish or clarify some links between QST and clinical symptoms manifested by NP patients.

NP is also an important issue during childhood and during adolescence, however many of the underlying aetiologies in which it occurs are uncommon or never encountered in children. However, several causes of NP have been described to date including traumatic injury, neurological and metabolic disease, inherited sensory nerve dysfunction, and CRPS. The evidence in basic and clinical studies of NP has disclosed significant age-related differences in clinical presentation and prognosis. Clinically, diagnosis, assessment and treatment of NP in children are based on methods, experience and evidence acquired from data in adults. Therefore, and due to the complexity when setting up any clinical trials involving children, it is especially important to increase the data available in children sharing every experience that can help clinicians to improve the knowledge when managing neuropathic states during childhood. The third, and last part of this work, focus its attention in the management of NP in children; particularly in the invasive management of CRPS.

MAIN OBJECTIVES

***First Study*: Altered potassium channel distribution and composition in myelinated axons suppresses hyperexcitability following injury.**

1. To determine neuroma' expression levels of Kv1.1 and 1.2 at juxtaparanode and paranode after traumatic nerve injury and its repercussion at the neurone' electrophysiological function and behavior.

***Second Study*: Symptom profiles in the painDETECT questionnaire in patients with peripheral neuropathic pain stratified according to sensory loss in quantitative sensory testing.**

1. To analyze if the overall painDETECT Questionnaire (PDQ) score or its items reflect phenotypes of sensory loss in neuropathic pain as determined by Quantitative sensory testing (QST) testing related pain thresholds in patients suffering from radiculopathy and fibromyalgia.
2. To investigate whether different types of sensory loss is present with different PDQ profiles, or if single PDQ items are sensitive to different types of sensory loss in clinical examination.
3. To analyze the possible correlation between QST obtained somato-sensory profiles of thermal and/or mechanical loss of function and PDQ profiles results of patients with neuropathic pain.

***Third Study*: Complex Regional Pain Syndrome in Children**

1. To examine the efficacy of a proposed multidisciplinary approach for the management of Paediatric Complex Regional Pain Syndrome in patients non-responders to conventional management.
2. To review the literature group experience looking for clinical evidence for invasive management in Paediatric Complex Regional Pain Syndrome in order to elaborate a guideline.

Chapter 1.

Altered potassium channel distribution and composition in myelinated axons suppresses hyperexcitability following injury.

1. Introduction

Following traumatic nerve injury spontaneous activity develops initially in myelinated and subsequently in unmyelinated sensory axons (Wall and Gutnick, 1974; Kajander and Bennett, 1992; Boucher et al., 2000; Michaelis et al., 2000; Wu et al., 2001; Liu et al., 2000a). The onset of this spontaneous activity is associated with the emergence of pain-related sensory changes in animal models and is critical for the maintenance of peripheral neuropathic pain (NP) (Haroutounian et al., 2014) in patients where selective blockade suggests the involvement of myelinated axons (Campbell et al., 1988). Ectopic activity is particularly prominent in myelinated afferents and peaks within the first few days post injury and then declines over subsequent weeks (Kajander and Bennett, 1992; Liu et al., 2000a; 2000b; Han et al., 2000). Such ectopic activity arises both at the neuroma site and also at the level of the dorsal root ganglion (DRG) (Han et al., 2000; Liu et al., 2000b; Amir et al., 1999; 2005; Wall and Devor, 1983).

Altered expression, function and trafficking of voltage-gated ion channels are key determinants of these excitability changes. Shaker type voltage-gated potassium channels (Kv1 channels) are important determinants of neuronal excitability. They are formed by heteromultimers of a and b subunits (MacKinnon, 1991). The characteristics of the outward currents they carry depend on subunit composition. Sensory neurons are known to express Kv1 channels and functionally these channels have been shown to limit excitability of sensory neurons: For instance Kv1.2 suppresses excitability at the level of the sensory neuron cell body (Gold et al., 1996; Rasband et al., 2001; Zhao et al., 2013; Everill et al., 1998) and Kv1.1 acts as a ‘brake’ on mechanosensitivity at the terminals of C- mechanonociceptor and Ab-mechanoreceptors (Hao et al., 2013). Kv1 channels also act as excitability brakes for cold thermal sensitivity in intact and damaged axons of primary sensory neurons (many of such fibres are also mechanosensitive) (Roza et al., 2006; Madrid et al., 2009). Kv1 channels are known to be expressed in the juxtaparanodal region of myelinated sensory axons. An unexplored issue, however, is whether the distribution of these channels changes under pathological neuropathic states.

Saltatory conduction in myelinated fibres depends on the molecular organization of channel domains within the axon (Chang and Rasband, 2013): voltage-gated sodium channels (Nav) are clustered at the node of Ranvier. Nodes are flanked by the paranode, which is an important point of attachment between the axon and the terminal loops of the Schwann cell. Just inside the innermost axoglial junction of the paranode is the juxtaparanode a domain enriched in Kv1 channels Kv1.1 and 1.2. The localisation of Kv1.1 and 1.2 to the juxtaparanode is dependent on the formation of a molecular scaffold, which includes the adhesion molecules caspr2 and TAG-1 (Poliak et al., 2003). In the naive state in adulthood, the juxtaparanodal Kv1 channels are thought not to have a major influence on axon conduction properties of peripheral myelinated axons (Poliak et al., 2003; Chiu and Ritchie, 1980; Sherratt et al., 1980; Rasband et al., 1998), probably because they are electrically insulated from the node of Ranvier under the myelin sheath. However during development (Vabnick et al., 1999) and following primary demyelination (Rasband et al., 1998) (during which myelin is removed but the axon remains intact), Kv1.1 and 1.2 become more widely distributed to include the paranode and even the node (Arroyo et al., 2004), and can act to suppress excitability. Although Kv1.1 and 1.2 expression within the soma is known to be down-regulated following axon transection, and this leads to hyperexcitability at the soma (Rasband et al., 1998; Ishikawa et al., 1999; Park et al., 2003), the distribution of these channels at the nodal complex and damaged nerve

terminal (in the neuroma that forms) has not been examined. Furthermore, little is known regarding the distribution of other members of the shaker type Kv1 channels family such as Kv1.4 and 1.6 following nerve injury.

Aim

Here, this work shows that within a neuroma, expression levels of Kv1.1 and 1.2 are markedly reduced but over time Kv1.4 and1.6 expression increases within juxtaparanodes and paranodes. At sites remote from injury, there is also a gradual redistribution of Kv1 channels to the paranode. Electrophysiological and behavioural experiments suggest that changes in subunit expression and redistribution of Kv1 channels act a 'brake' on the hyperexcitable state that arises in myelinated axons following traumatic nerve injury.

2. Materials and Methods

Animals and surgery

Adult male Sprague-Dawley rats were used in accordance with UK Home Office and Pontificia Universidad Catolica's regulations (animals in the UK were purchased from Charles-River UK, animals from Chile were purchased onsite). Rats were group housed and placed on a 12 hour-light 12 hour-dark cycles. Two different nerve injury models were used: the neuroma model and the L5 spinal nerve transection (SNT) model. The neuroma model of NP was based on the TNT model (Dorsi et al., 2008), but performed with some modifications. Briefly, the sciatic nerve was dissected free of adjacent tissue, ligated with a suture, and cut proximal to its bifurcation. The needle from the suture was passed through a subcutaneous tunnel to the lateral aspect of the hindlimb where it was pushed through the skin. The nerve was drawn into the tunnel until the ligature is adjacent to the skin. The suture was cut, and the incision closed. The suture tied to the distal end of the sciatic nerve was visible through the skin and served as the target for mechanical stimuli. An analogous site served as the target on the contralateral hindlimb. For the L5 SNT model (Kim and Chung, 1992), one-third of the L6 transverse process was removed and the L5 spinal nerve was identified and dissected free from the adjacent L4 spinal nerve and then tightly ligated using 6–0 silk and then transacted distally to the suture. Sham-operated animals served as a control. We used these two different models as the neuroma model is the most adequate for performing behavioural tests as the injured nerve can be directly stimulated, while the L5 SNT model has the advantage that it gives certainty that all the dorsal root axons studied had their peripheral terminals injured. For both models animals were deeply anaesthetised with a mix of isofluorane and oxygen. Postoperative analgesia was given for the first 5 days postop (tramadol 50 mg/kg/day p.o). Animals were checked every day after surgery to check for self-mutilation behaviour (autotomy), which prompted us to sacrifice the animal. Calculation of the sample size needed was done for each experiment as described below. Experimental protocols were reviewed and approved by 'Coordinación de Ética, Bioética y Seguridad de las investigaciones UC' (experiments done in Chile) and were performed in accordance with the UK Home Office regulations (experiments done in the UK). We report this study in compliance with the ARRIVE guidelines (Kilkenny et al., 2010) (20 points checklist).

Patients and controls

The study was conducted at Hospital Clínico UC-Christus in Santiago, Chile. Morton's neuroma patients that were due surgery for resection of painful neuromas were recruited for donating a small sample of the tissue resected during surgery. Control samples were obtained from subjects undergoing hand reconstructive surgery in where the sural nerve is harvested and used as a bridge to connect disrupted ends of motor nerves in the hand. A small sample for these healthy sural nerves was collected to use as control in this study. We used the Numeric Rating Scale (NRS; which is a self-reporting scale where 0 is no pain and 10 is the worst imaginable pain) to assess for pain before surgery. Informed consent was obtained from all subjects before surgery. The study protocol was assessed and approved by the Ethics Scientific Committee of the School of Medicine Pontificia Universidad Católica de Chile. The sample sizes were calculated using a power of 80% and an a error of 0.05%, assuming a 2 times increase or decrease in Kv channel expression with a variance of 0.6 from the mean, which resulted in a sample needed of 3 patients per group.

Histology

After a defined survival time (7 and 21 days), animals were terminally anaesthetized with pentobarbital and transcardially perfused with 0.9% heparinised saline. The L5 DRG, the L5 spinal nerve, and the sciatic nerve were removed. We dissected the sciatic nerve free from connective tissue and collected 5 mm from the site of the neuroma and 5 mm from a site 1 cm proximal. Tissue for immunohistochemistry was post fixed in 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB) for 30 min and cryoprotected in 20% sucrose for 3 days. Tissue obtained from patients was fixed immediately after resection in 4% PFA for 30 min and then cryoprotected in 20% sucrose for 3 days. The samples were embedded in OCT, cryostat cut (8 mm) and thaw-mounted onto glass slides. Sections were pre-incubated in buffer (PBS, pH 7.4, containing 0.2% Triton X-100 and 0.1% sodium azide) containing 10% normal donkey serum for 30 min and then incubated with primary antibodies over-night at room temperature. Primary antibodies used are shown in Table 1. Following primary anti- body incubation, sections were washed and incubated for 2 hrs. with secondary antibody solution (donkey anti-rabbit Cy3 1:400; goat anti guinea pig AMCA 1:100, donkey anti mouse FITC 1:400; all from Stratech, UK). Slides were washed with PBS, cover-slipped with Vectashield mounting medium (Vector Laboratories, UK) and visualised under a Zeiss Axioplan 2 fluorescent microscope (Zeiss, UK). All quantification of different IHC parameters was done with the investigator blinded to the identity of the group to which the animals belonged. Nodal quantification was done by assessing on average 31 nodes per animal, and using 4–5 animals per condition. For quantification of bII spectrin the intensity of the immunofluorescence of the axonal paranodal area (identified by caspr staining) was measured and the background of each section was subtracted. Then, measurements were normalised against the mean of the controls (naıve axons). The sample sizes were calculated using a power of 80% and an a error of 0.05%, assuming a 2 times increase or decrease in expression with a variance of 0.5 from the mean, which resulted in a sample needed of 4 animals per group.

We quantified sodium channel clusters following the following criteria: (1) typical nodes were nodes where the Nav channels fill the gap at the node of Ranvier as identified by the paranodal staining of caspr on both

sides of the node, (2) split nodes were nodes that had two distinct Nav channels accumulations, separated by a gap in the Nav channel staining within the same fibre and with each Nav channels accumulation flanked on one side with caspr staining, or (3) heminodes were nodes where the caspr staining located on only one side of a contiguous Nav channel accumulation, (4) while those Nav channel accumulations lacked an association with caspr were classified as 'naked' accumulations.

Western blot analysis

Tissue was collected, quickly frozen in liquid nitrogen and was homogenized in NP40 lysis buffer (20 mM Tris, pH 8, 137 mM NaCl, 10% glycerol, 1% NP-40, 2 mM EDTA), 20 mM leupeptin, 5 mM sodium fluoride, 1 mM sodium orthovanadate, 1 mM PMSF and protease inhibitor cocktail). The lysate were spun at 13,000 rpm at 4 ◦C for 15 min and the protein concentration of supernatant was determined using a BCA Protein Assay kit (Thermo Scientific). 50 mg of each sample was separated using 8% or 10% SDS-PAGE, and transferred to nitrocellulose membranes. Membranes were then blocked in 10% skimmed milk in PBS-T (PBS+ 0.1% Tween 20) for 1 hr at room temperature. Membranes were incubated with primary antibody (anti mouse Kv1.1, Kv1.2, Kv1.4, Kv1.6, GAPDH, PGP9.5 and anti-rabbit Caspr2 as shown in Table 1), diluted in PBS-T at 4 ◦C overnight. After washing with PBS-T for 6 times and5 min each time, membranes were incubated with sheep anti-mouse or donkey anti rabbit HRP-conjugated secondary antibody (1:10,000–1:20,000; ECL, GE Healthcare, Amersham, UK) at room temperature for 1 hr. After several PBS-T washes as described above membranes were revealed using ECL-prime reagent (GE Healthcare) for 5 min for detection by autoradiography.

For WB of Kv1.4 and Kv1.6 in rat tissue (sciatic nerve and DRG) the membranes were cut in three pieces; the top piece was probed with Kv1.4 antibody, the middle one was probed with Kv1.6 anti- body and the bottom one probed with GAPDH antibody. For WB of Kv1.1 and for Kv1.2 the membranes were cut in 2 pieces: the top one was probed with either Kv1.1 or Kv1.2 antibody and the bottom one was probed with GAPDH antibody. The 2 or 3 pieces of the membranes were lined up as a single membrane before exposing it to the film so that the molecular weight can be calculated by measuring the running distance of the molecular weight marker and the target bands. This could be done as the bands labelled by the antibodies have quite different molecular weights. This allowed us to optimize the use of the tissue obtained from animals and reduce the number of animals needed (in accordance with our obligations under animal licensing procedures).

Quantification and analysis

For Western Blots analysis, films were scanned with Cannon Scanner (LiDE 210), and the intensity of specific bands was quantified using Quantity One software (Bio-Rad). The same size rectangle was drawn around each band to measure intensity, and the background was subtracted. Target band detected was normalized against loading control GAPDH or PGP9.5 correspondingly for analysis. The sample sizes were calculated using a power of 80% and an a error of 0.05%, assuming a 2 times increase or decrease in expression with a variance of 0.5 from the mean, which resulted in a sample needed of 4 animals per group (we used 6 animals per group in case we had to put any animal down due to autotomy).

Table 1.

Antibody	Concentration used		Company
	IHC	WB	
Rabbit anti Pan voltage gated sodium channel (Cat No. S6936)	1:1000		Sigma-Aldrich
Mouse anti Kv1.2 (K14/16.2)	1:100	1:500	UC Davis/NIH NeuroMab Facility
Mouse anti Kv1.1 (K36/15.1)	1:100	1:200	UC Davis/NIH NeuroMab Facility
Mouse anti Kv1.4 (K13/31)	1:100	1:200	UC Davis/NIH NeuroMab Facility
Mouse anti Kv1.6 (K19/36)	1:100	1:500	UC Davis/NIH NeuroMab Facility
Guinea Pig anti Caspr	1:1000	1:1000	From Dr Manzoor Bhat - UT Health Science Center San Antonio (PMID: 11395000)
Rabbit anti Caspr2 (ab105581)	1:500	1:400	Abcam
Rabbit anti Pan Neurofascin	1:500		Gift from Prof Peter Brophy-University of Edinburgh (20129933)
Mouse anti βII spectrin (Clone 42)	1:500	1:1000	BD Bioscience

Table 1: Different antibodies used in the study. IHC: Immunohistochemistry WB: Western Blot analysis.

Electron microscopy

Sciatic nerves were dissected at the site of the neuroma and were processed for resin embedding as previously described (Huang et al). Briefly nerves were post fixed in 3% glutaraldehyde at 4Cº over-night, washed in 0.1 M PB, osmicated, dehydrated, and embedded in epoxy resin (TAAB Embed- ding Materials, UK). Longitudinal sections 1 mm thick were cut on a microtome and stained with toludine blue before being examined on a light microscope. Ultrathin sections were cut on an ultra-microtome and stained with lead uranyl acetate. Sections were mounted on unsupported 100 mesh grids. Sections were visualised on a PHILIPS TECNAI 12 BIOTWIN transmission electron microscope at the Unidad de microscopia avanzada, Pontificia Universidad Católica de Chile. We measured the diameter of the axons at the site of the node, the maximal and minimal distance between interloops, the distance between the glia and the axon, the number of detached loops, and the number of everted loops using Image J (NIH, USA) and a 135000x magnification. We quantified between 8 and 14 nodes per animal, and we used 5 animals per condition (sample sizes were calculated using a power of 80% and an a error of 0.05%, assuming a change in distance of 50% with a variance of 0.4, which resulted in a sample needed of 4 animals per group, however due to the difficulty in the technique we included one more animals in each group). The investigator was blinded to the treatment group of each specimen, however, this was sometimes difficult to conceal as the anatomy in the injured nerves was much more disrupted than in naive nerves.

In vivo electrophysiological recording

Recordings were performed under anaesthesia (urethane, 1.5 g/Kg, i.p.) on naive rats (n = 10, 222 neurons), or after sciatic nerve ligation at 2 days (n = 4, 291 neurons), at 7 days (n = 7, 241 neurons), and at 21 days (n = 7, 237 neurons). A tracheotomy was performed and the L5 dorsal roots and DRGs were exposed via laminectomy. Sciatic nerve neuroma with proximal nerve (5–6 mm long) and contralateral uninjured sciatic nerve were exposed. The contralateral sciatic nerve was acutely cut to disconnect from the periphery just before recording. The entire site was covered in agarose gel and four chambers created by removing blocks of this gel. These were 1) neuroma chamber, containing ipsilateral neuroma and part of sciatic nerve which is subjected to stimulation; 2) acutely cut nerve end chamber, containing contralateral sciatic nerve proximal end; 3) DRGs chamber, containing L5 DRGs from both sides; 4) spinal recording chamber, containing part of L5 dorsal roots from both sides near entry zone to spinal cord. The neuroma chamber and nerve cut end chamber were filled with mineral oil during stimulation, and the oil was replaced with aDTx (100 nM in saline) during toxin application. The DRGs chamber was filled with saline or aDTx/saline solution, and the recording chamber was always filled with mineral oil. The pool temperatures were not controlled, but as animals were warmed using an infrared lamp from the back, the pool was therefore heated, and typically was at 34–35Cº. Just before recording, the L5 dorsal root was cut near entry zone, a filament was teased out and hooked up for recording. Each filament was stimulated electrically with increasing current to recruit sequentially each conducting axon in that filament. The conduction velocity of each conducting axon was noted. Thus, the number of functioning axons in each filament was deter- mined (typically, 6–10). Spike discrimination was used to detect the number different axons firing spontaneously in each filament (typically 0–3) during a pre-treatment baseline and under 3 different treatment conditions: 1) no aDTx in any of the chambers; 2) aDTx in neuroma or nerve cut end chamber; 3) aDTx in neuroma or nerve cut end chamber and DRGs chambers. The aDTx was applied for at least 20 min before recording. An independent investigator prepared the drugs individually and labelled

them for each animal according to the randomization schedule. Data analysts were blinded as the conditions under which all recording were made.

Signals were amplified with an AC-coupled amplifier (Neurolog NL104A with headstage NL100AK), then high-pass and low-pass filtered (Neurolog NL125) at 500 Hz and 5 KHz frequencies. The filtered signals were passed through a Humbug 50 Hz noise eliminator (Quest scientific, Vancouver, BC, Canada), further amplified (Neurolog NL 106), fed to an analog-to-digital converter Power- Lab, and sampled at 20 KHz with Labchart software (ADinstruments, UK). Stimulation (200 ms square-wave pulses) was delivered from a stimulus isolator (Neurolog, NL800A). The filter settings used strongly favours recordings from A-fibres and not C-fibres. All the fibres recorded to nerve stimulation conducted in the A fibre range (>2 m/sec). The size of the filaments recorded was also unfavourable for detecting clear single unit C fibre activity.

Three minutes baseline was recorded to examine spontaneous activity. The percentage of spontaneously firing units was calculated as the number of spontaneously active units divided by the number of conducting fibres determined in recruitment recording. The firing rate was calculated as the total number of spikes during recording divided by the time recorded. The mean firing rate per unit was the firing rate divided by the total number of different units recorded in each treatment group.

For the axonal mechanosensitivity experiments (n= 4,259 neurons), mechanical stimulation was applied to the neuroma using increasing forces of von Frey filaments (4, 8, and 15 g), and the number of distinct spikes (neurons) firing in response were counted following spike discrimination. The total number of conducting axons in each filament was determined by incremental electrical stimulation of the sciatic nerve. The percentage of mechanosensitive units was calculated as the number of different neurons responding by firing action potentials upon mechanical stimulation, divided by the number of conducting fibres (which was determined in the same way as for the spontaneous activity experiments). Axonal mechanosensitivity was assessed before and after toxin application at 21 days after axotomy. Mechanosensitivity experiments were carried out on separate animals to spontaneous activity experiments to ensure that any spontaneous activity encountered was not caused acutely by the repeated mechanical stimulation of the neuroma.

Data was analysed using software Labchart. Statistics comparing proportions of neurons exhibiting either spontaneous activity or mechanosensitivity were performed using chi-square test with Yates correction. Values were reported as percentages, calculated from the proportions.

Assessments of mechanical sensitivity

Mechanical withdrawal thresholds were assessed by applying a range of Von Frey hairs (Somedic, Sweden) to the skin over the neuroma site (labelled with a suture as previously described). Animals were randomised to receive either subcutaneous aDTX (0.5 ml at 100 nM in saline, Alomone, UK) or saline (which was administered locally at the site of the neuroma) using a computer-generated random sequence. The sample sizes were calculated using a power of 80% and an a error of 0.05%, assuming a 60% decrease in withdrawal threshold with a variance of 25% from the mean, which resulted in a sample needed of 7 animals per group. Experimental groups were the following: base- line with vehicle, baseline with toxin; day 3 after surgery with vehicle, day 3 with toxin; day 7 with vehicle, day 7 with toxin; day 21 with vehicle, day 21 with toxin. To reduce the amount of animals of the study the animals that received

saline only were used again for the consecutive time-points. The animals that received toxin had to be sacrificed after testing, as the toxin is irreversible. The toxin or saline were injected 30 min before testing. Autotomy after nerve injury (especially neuroma model) appears at around 10 days after injury. Therefore, we allocated 2 extra animals for the saline group, and 2 extra for toxin day 21. We had to sacrifice 1 animal from the saline group at day 6, and 2 animals from the toxin group day 21 (at day 15 and 17 after injury respectively), due to self-mutilating behaviour. For testing, rats were gently restrained using a towel on a table. Calibrated von Frey hairs were applied to the skin covering the neuroma until the fibre bent. Withdrawal of the limb by the animal was recorded as a response. The 50% withdrawal threshold was determined using the up- down method (Dixon, 1980). An independent investigator prepared the drugs individually and labelled them for each animal according to the randomization schedule. Operators and data analysts were blinded throughout the study. The data were distributed normally and the differences between groups was analysed using a 2 way ANOVA repeated measures. Values were reported as mean ± SEM.

This experiment was repeated for testing CP339818 (Kv1.4 blocker; #C-115, 0.5 ml at 300 nM in saline Alomone Labs UK). We randomly allocated 8 animals per group, and we had to put one animal from the saline group down due to autotomy at day 12.

Ethics

Human subjects: Informed consent, and consent to publish, was obtained from all subjects to collect and analyse nerve samples before surgery. Subjects underwent surgery by indication of their physician and samples were obtained from biological tissue that was otherwise due to be incinerated. The study protocol was assessed and approved by the Ethics Scientific Committee of the School of Medicine Pontificia Universidad Católica de Chile (reference number 14-389).

Animal experimentation: This study was performed in strict accordance with UK Home Office and Pontificia Universidad Católica's regulations. Experimental protocols were reviewed and approved by "Coordinación de Ética, Bioética y Seguridad de las investigaciones UC" (experiments done in Chile- Protocol CBB230/2013) and were performed in accordance to the UK Home Office regulations (experiments done in the UK). We report this study in compliance with the ARRIVE guidelines (20 points checklist).

3. Results

Expression of Kv1 channels subunits switches at nodal regions after nerve injury .

To investigate the role of Kv1 channels in hypersensitivity after nerve injury we used a model of complete sciatic nerve transection followed by positioning of the proximal stump superficially under the skin of the leg [modified version of Dorsi et al. (2008)]. This model enables us to study both the expression of Kv1 channels within the neuroma and undertake behavioural analysis using specific blockers of Kv1 channels.

To study how the localisation of Kv1 channels changes within the nodal complex, we used a pan-Nav channel antibody to label the node of Ranvier, a Caspr antibody to label the paranode and Kv1.2 and Caspr2 antibodies to label the juxtaparanode. In the naıve axon, we observed that over 90% (mean 91 ± SEM 2.9%, n = 4 animals) of the nodes presented a characteristic morphology with Nav clustering in the centre, surrounded by caspr at both sides and Kv1.2 clustered within the juxtaparanode. Other members of the Kv1 channels family, Kv1.4 and 1.6 are expressed by DRG cells (Everill et al., 1998; Thakur et al., 2014; Chiu et al., 2014). We found that in the naıve state Kv1.4 was only expressed within a very small proportion of nodes (5.5 ± 4.6%, n = 4 animals) within the juxtaparanode and Kv1.6 was not present within the nodal complex (Figure 1).

Figure 1.

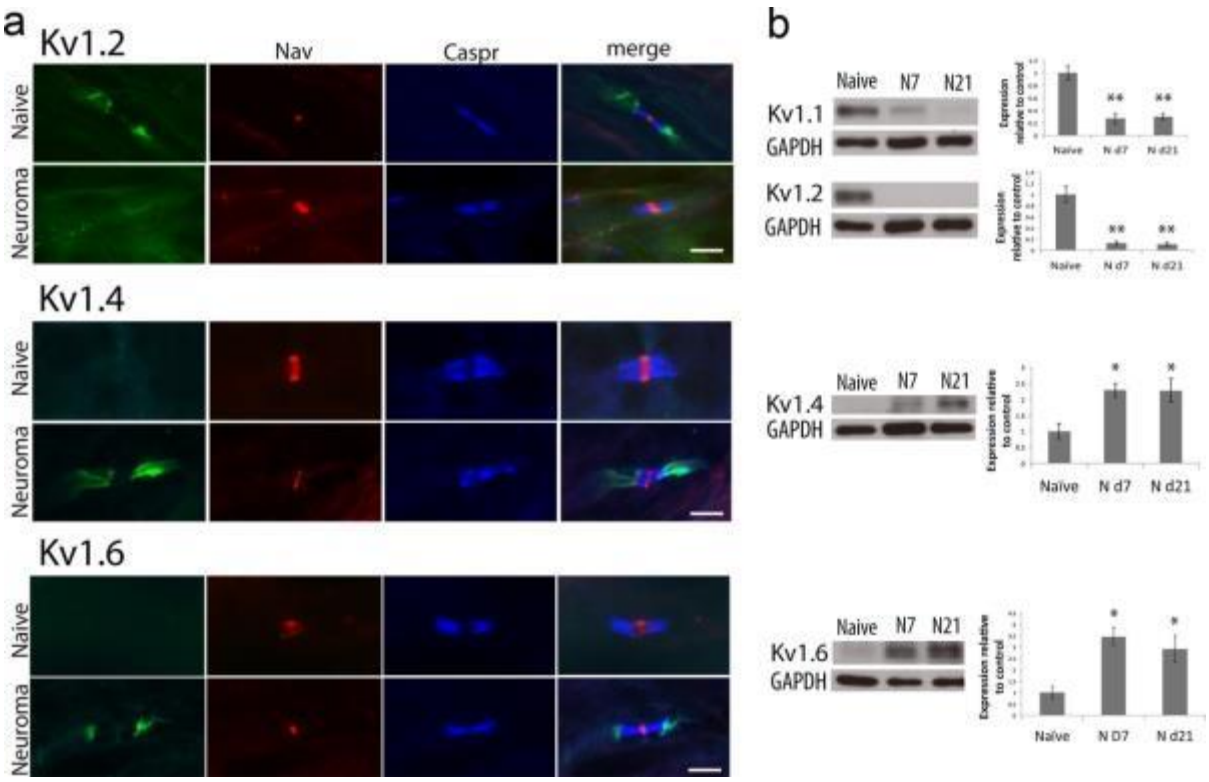


Figure 1. Kv1 channels expression in the naive nerve and 21 days after sciatic nerve axotomy (note that the samples were collected from the neuroma site). (a) Representative images of longitudinal nerve sections immunostained with Kv1 channels in green (Kv1.2, Kv1.4 and Kv1.6 respectively), a panNav antibody in red (to identify the node), and caspr in blue (to identify the paranode). Kv1.2 is expressed in the juxtaparanode in naıve nerves but it is not present at 21 days after nerve injury. Kv1.4 and kv1.6 are not present in uninjured nerve but are expressed after nerve injury. Note that when Kv1.4 and Kv1.6 are expressed, they are not confined to the juxtaparanode only but invade the paranode. (b) Western blots showing expression of Kv1 channels in the naıve nerve, at 7 and 21 days after axotomy. Kv1.1 and Kv1.2 are expressed in the naıve nerve and down-regulated after axotomy, while Kv1.4 and Kv1.6 have a low/null expression in the naıve nerve and are up-regulated after injury (*p<0.05, **p<0.001, one Way ANOVA, n=6 per group). Scale bars = 5 mm.

At the site of the neuroma (day 7 and 21), only half of the nodes demonstrated this typical morphology (day 7 = 47.5 ± 5.1%, day 21 = 46 ± 6%, n = 4 animals per group, 36–64 nodes per animal); the rest were split (day 7 = 23.3 ± 5.9%, day 21 = 13.5 ± 2.7%), presented as heminodes (caspr at one side only, day 7 = 21.8 ± 4.4%, day 21 = 28.9 ± 5.5%), or were 'naked' (Nav clusters alone, with no caspr, day 7 = 7.2 ± 3%, day 21 = 11.4 ± 3.2%, Figure 2). This is in accordance with previous literature examining the localisation of voltage-gated sodium channels (Henry et al., 2006; Thakur et al., 2014). At day 7 after injury, Kv1.2 channels were not located strictly not only in the juxtaparanodal regions but also overlapped with paranodal proteins. To objectively measure this, we quantified the distance between the Nav channels staining and the distal end of the caspr staining, and the distance between the Nav channels staining and the proximal end of Kv1 channels. The difference between these two distances was indicative of the level of overlap between Kv1 channels and paranodal proteins (note that 'naked' nodes were not included in the analysis of the spatial distribution of Kv1 channels because by definition these only consist of Nav clusters without paranodal and juxtaparanodal proteins). In naıve axons the distance between Nav channels staining and the end of caspr was 3.8 ± 0.2 mm, and the distance between Nav channels staining and the start of Kv1.2 staining was 4.2 ± 0.2 mm, resulting in a relatively small, albeit positive, difference between these distances (0.5 ± 0.08 mm, n = 4 animals, 25–40 nodes per animal), indicating there was no overlap. At day 7 after injury, the distance between Nav channels staining and the end of caspr was 4 ± 0.1 mm, and the distance between Nav channels staining and the start of Kv1.2 staining was 3.2 ± 0.2 mm, giving a negative value for the difference between both distances (-0.8 ± 0.1 mm, n = 3 animals, 32–35 nodes per animal), which indicates that the Kv1 channels were co-localised with caspr staining and moving closer to the node (Figure 3). Note that the distance between Nav channels staining and the end of caspr staining remained unchanged after injury, while the distance between Nav channels staining and the start of Kv1.2 staining was significantly reduced. Contactin-associated protein-like 2 (Caspr2) forms a complex with Kv1 channels at the juxtaparanode (Chiu et al., 2014). We evaluated if caspr2 moves closer to the node together with Kv1 channels. We measured the distance between the Nav channels staining and the distal end of the caspr staining, and the distance between the Nav channels staining and the proximal end of caspr2. In naıve axons, the distance between Nav channels staining and the end of caspr was 3.8 ± 0.3 mm, and the distance between Nav channels staining and the start of caspr2 staining was 4.3 ± 0.0 mm, resulting in a small difference between these distances (0.49 ± 0.09 mm, n = 4 animals, 25–30 nodes per animal), indicating there was no overlap. At day 7 after injury, the distance between Nav channels staining and the end of caspr was 4.1 ± 0.2 mm, and the distance between Nav channels staining and the start of caspr2 staining was 2.8 ± 0.2 mm, giving a negative value for the difference between both distances (-1.2 ± 0.1 mm, n = 3 animals, 30 nodes per animal), which indicates that the caspr2 co-localised with caspr staining and had moved closer to the node together with Kv1 channels (Figure 3).

Figure 2.

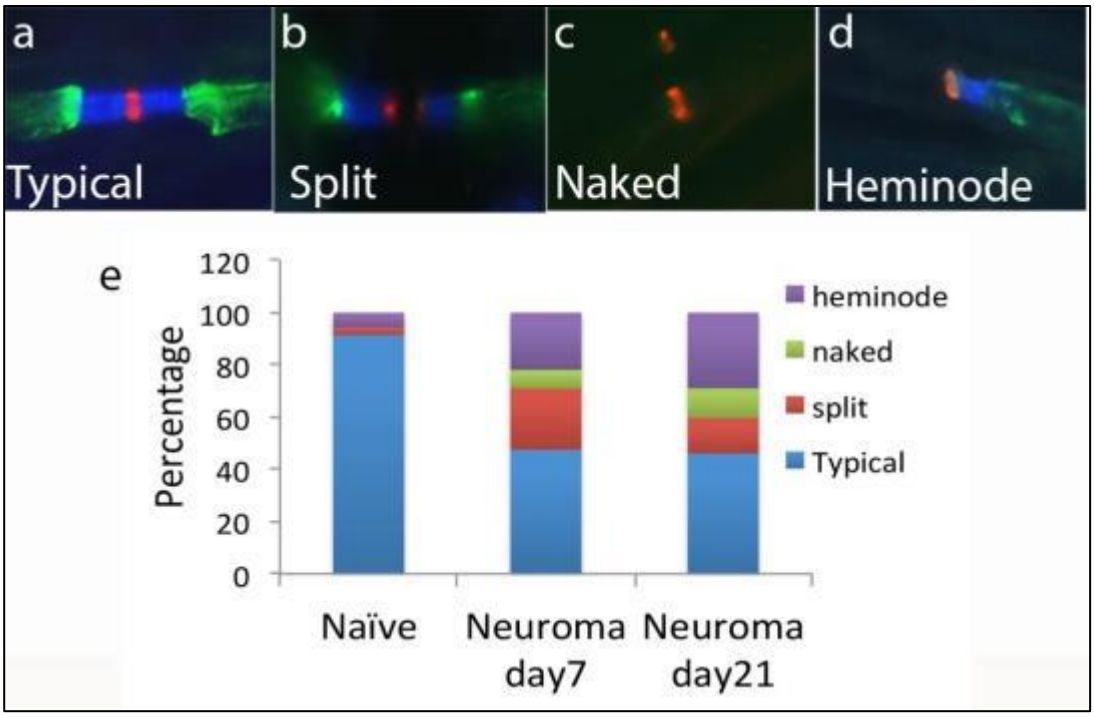


Figure 2. Nav channel expression.

Representative sections of longitudinal nerves immunostained with Kv1.2 in green, a panNav antibody in red (to identify the node), and caspr in blue (to identify the paranode) from neuroma day 21. (a) A typical pattern of Nav expression localized at the node of Ranvier and flanked by caspr staining is shown. The altered forms of Nav channel accumulations seen in the injured nerve included (b) split nodes: These were nodes that had two distinct Nav channels accumulations, separated by a gap in the Nav channels staining within the same fibre and with each Nav channels accumulation flanked on one side with caspr staining, or (c) naked nodes: those Nav channel accumulations that lacked an association with caspr (d) heminodes: nodes where the caspr staining was located on only one side of a contiguous Nav channel accumulation. (e) Quantification of different types of sodium cluster accumulation in the naïve state and after nerve injury is shown.

Figure 3.

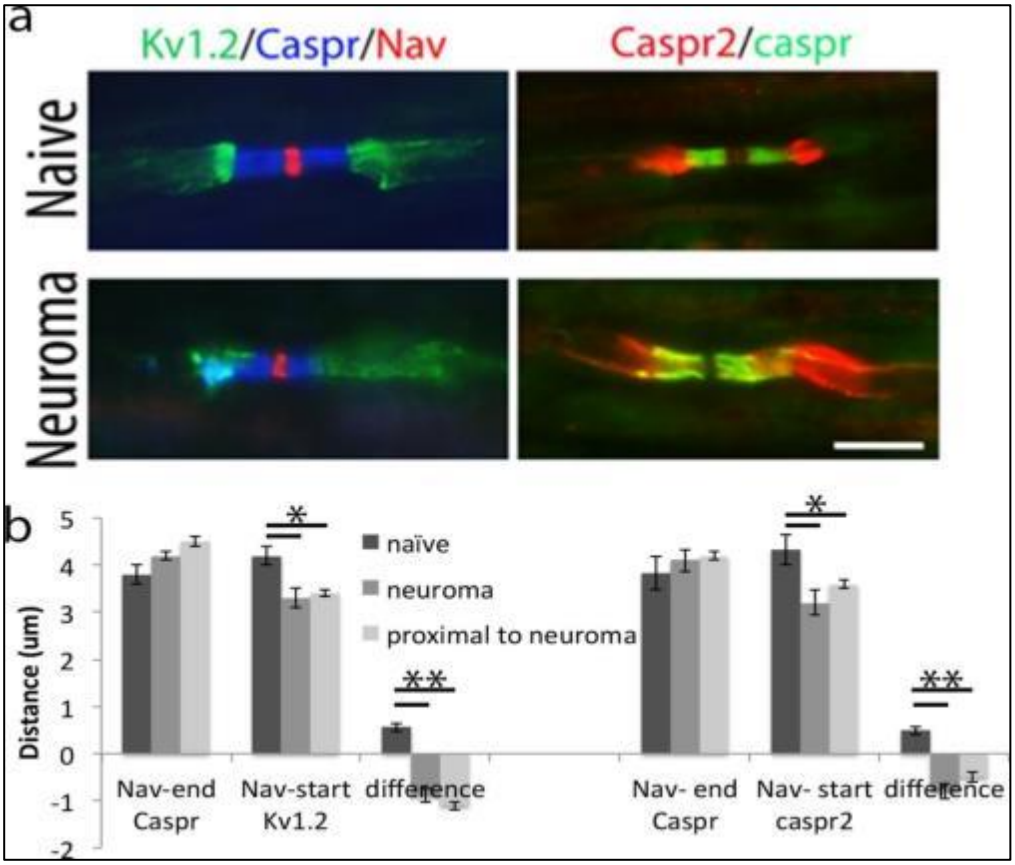


Figure 3. Relocalisation ok Kv1.2 and caspr2 at 7 days after neuroma.

(a) Representative longitudinal sections of nerves immuno-stained with Kv1.2 in green, a panNav antibody in red (to identify the node) and caspr in blue (to identify the paranode). Kv1.2 is expressed in the juxtaparanode in naïve nerves but it also co-localized with caspr staining at 7 days after injury. Representative longitudinal sections of nerves immuno-stained with caspr2 in green and caspr in red. Caspr2 is confined to the juxtaparanode in naïve nerve but co-localized with caspr at 7 days after injury. (b) We quantified the distance between the sodium channel staining (Nav) and the end of the caspr staining, distance between the sodium channel staining (Nav) and the start of the Kv1.2/1caspr2 staining, and difference between these distances. A negative value represents an overlap of paranodal and juxtaparanodal proteins. Note that he distance between the sodium channel staining (Nav) and the end of the caspr staining remains unchanged after nerve injury, while the distance between the sodium channel staining (Nav) and the start of the Kv1.2/caspr2 staining is significantly shortened after nerve injury, indicating co-localization of Kv1.2 and caspr2 with caspr (n = 5 animals, 20–41 nodes per animal), $p<0.001$, one way ANOVA Tukey post hoc tests). We analysed uninjured (naïve) nerve, nerve at the site of the neuroma (day 7), and nerve 1 cm proximal to the neuroma (day 7). The effect on Kv1.2 re-localization remains the same at the site far from the neuroma. The effect on caspr2 re-localization is slightly smaller at the site 1 cm proximal to the neuroma compared with the neuroma site, but it is still significantly different from the naïve $^{**}p<0.001$, $^{*}p<0.05$, PRN = paranode, JXP = juxtaparanode. Scale bars = 5 μm.

The redistribution of the channels seen could simply be a reflection of direct injury at the site of axotomy. We therefore, studied a site proximal to the neuroma (1 cm) and compared it to the neuroma site. The effect on Kv12 re-localization remains the same at the site far from the neuroma: The difference between Nav channels staining and the end of caspr distance and Nav channels staining and the start of Kv1.2 distance was -1 ± 0.09 mm, at the site close to the neuroma and -1.1 ± 0.09 mm, at the site far from the neuroma ($p = 0.6$, n = 5 animals, 24–26 nodes per animal). The effect on caspr2 relocalization is slightly smaller at the site 1cm proximal to the neuroma compared with the site close to the neuroma, but it is still significantly different from the naıve ($p<0.001$): The difference between Nav channels staining and the end of caspr distance and Nav channels staining and the start of caspr2 distance was -1 ± 0.1 mm, at the site close to the neuroma and -0.5 ± 0.1 mm, at the site far from the neuroma ($p = 0.06$, n = 5 animals, 21–26 nodes per animal, Figure 3c). This change on juxtaparanode proteins localisation could be due to a disorganisation of the paranodal axoglial junctions (paranode loops). Therefore, we examined their ultrastructural anatomy using electron-microscopy and measured the distance between the axon and the paranodal loops (axoglial distance), the number of detached and everted loops and the minimal and maximal distance between loops. We analysed sciatic nerves from sham-operated and neuroma animals and we observed very few detached or everted loops, with no differences between groups. The close apposition between the axon and the paranodal loop was unchanged as the axoglial distance was not significantly changed (sham = 9.7 ± 0.4 nm, neuroma = 11.4 ± 0.7 nm, n = 4–5 animals per group, 6– 9 nodes per animal one way ANOVA $p = 0.08$). We observed a small but significant increase in the maximal distance between loops (sham = 12.5 ± 1 nm, neuroma = 18.9 ± 1.4 nm, one way ANOVA, $p = 0.005$). In summary, these results suggest that although there was no major disruption of the septate axoglial junctions there was a small but significant increased separation between the paranodal loops (Figure 4). Note that the axonal diameter at the node did not change (Figure 4d).

bII spectrin is a cytoskeletal protein that has recently been shown to be essential for the localization of Kv1 channels to the juxtaparanode and is proposed to form a sub-membranous barrier to lateral diffusion of Kv1 channels into the paranode (Zhang et al., 2013). Using IHC, we looked at bII spectrin in the neuroma and found that it is expressed at the paranode and juxtaparanode. We quantified the staining at the paranodal domain and found that the expression of this protein was reduced by more than half compared to naive (immunofluorescence normalised to naıve: 0.4 ± 0.02, $p<0.001$, t-test, n = 50–83 heminodes, a–b). bII spectrin is also expressed in the sub-membranous regions of Schwann cells where it does not appear to change with nerve injury. To further quantify the change of expression of this protein in the neuron, we performed Western blotting in the soma of sensory neurons (dorsal root ganglia- DRG) and observed a 30% decrease following nerve injury compared to naive (expression relative to naıve: 0.7 ± 0.08, $p = 0.04$, t-test, n = 4, Figure 5c–d).

At day 21 after injury, in marked contrast with the naıve axons, very few of the nodes at the neuroma site (day 21) showed Kv1.2 immunostaining (8.3 ± 0.8% in neuroma vs. 86.1 ± 4.4% in naive, n = 4 animals per group, 25 nodes per animal $p<0.001$ t-test). Conversely, most of the nodes at the neuroma site (day 21) showed intense Kv1.4 immunostaining (73.3 ± 12% in injured vs. 5.5 ± 4 in naive, n = 4 animals per group, 30 nodes per animal $p<0.001$ t-test) and Kv1.6 immunostaining (66.6 ± 14% vs. in injured vs. none in naive, n= 4 animals per group, 30 nodes per animal $p<0.001$ t-test) (Figure 1).

Figure 4

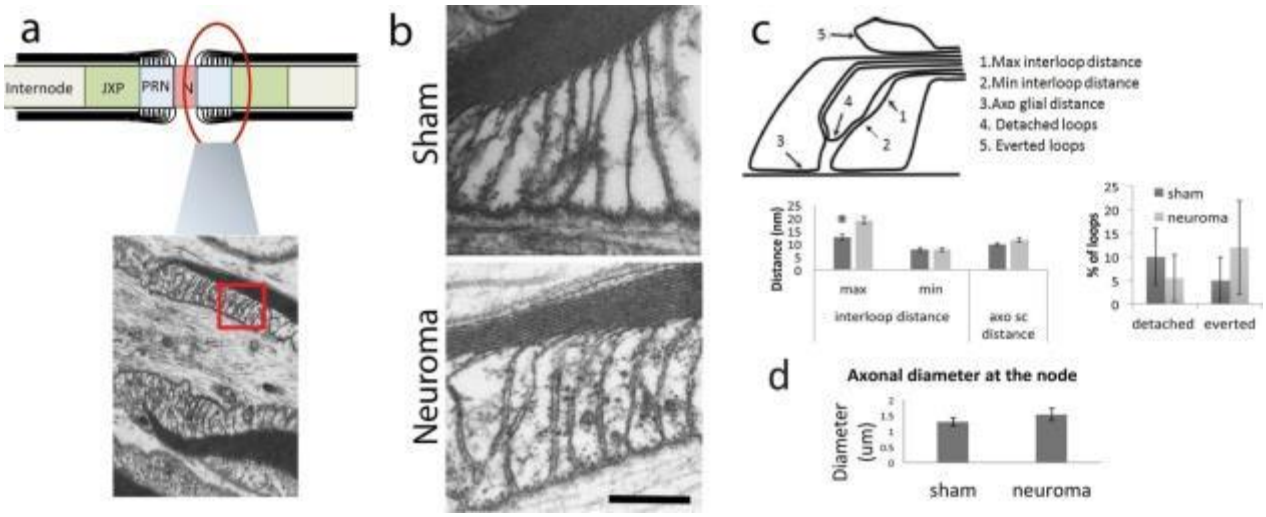


Figure 4. Ultrastructural anatomy of the node of Ranvier within the sciatic nerve following axotomy. We used electron microscopy to look at the ultrastructure anatomy of the node. (a) Shows a diagram of the node, paranode and juxtaparanode and a low magnification section of this area in a sham-operated nerve. The red box denotes the area that was used for quantification as seen in b. (b) High magnification views of the paranodal loops are shown in the sham and 21 days following axotomy (magnification 135 000x) (c) We quantified different aspects of the attachment of the Schwann cell paranodal loops to the axon. This is illustrated in right panel which denotes the different parameters measured: The maximal and minimal distance between interloops, the distance between the glia and the axon, the number of detached loops and the number of everted loops. We found a significant increase in the maximal distance between loops in the neuroma compared to sham nerves (one way ANOVA, $p = 0.005$). There were no significant differences in any of the other measurements. (d) We quantified the diameter of the axons at the site of the node and found no difference between the uninjured and injured axons. Scare bars: 200 nm

Figure 5.

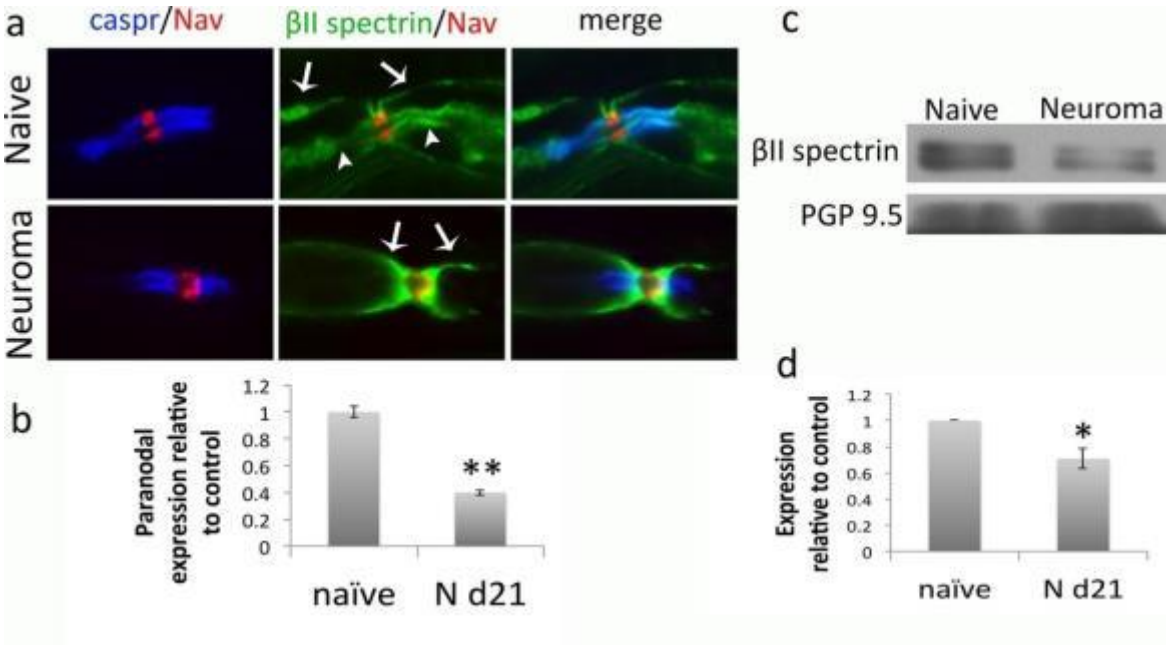


Figure 5. βII spectrin expression in naïve and neuroma nerves.

(a) Representative sections of longitudinal nerves immunostained with βII spectrin in green, a panNav antibody in red (to identify the node), and caspr in blue (to identify the paranode). βII spectrin is expressed both in the surface of Schwann cells (arrows) and in the axon at the paranodal and juxtaparanodal region (arrow heads) in naïve nerves. At 21 days after axotomy (neuroma), βII spectrin can be only seen in the Schwann cell (arrows) but not in the axonal domains. (b) Quantification of βII spectrin immunofluorescence in the paranode (identified by caspr staining) showing a significant reduction in neuroma versus naïve (immunofluorescence normalised to naïve: 0.4 ± 0.02, $p<0.001$, t-test, n = 50–83 heminodes). (c) Western blots showing expression of βII spectrin in the DRG of naïve and day 21 neuroma. (d) Quantification of WBs. Expression of βII spectrin in the DRG was reduced by 30% after nerve injury. PGP9.5 was used as a loading control (expression relative to naïve: 0.7 ± 0.08, $p = 0.04$, t-test). **$p<0.001$, *$p<0.05$. Error bars denote SEM.

We used Western blotting to quantify the expression of the different a subunits and found that Kv1.1 and Kv1.2 expression were significantly reduced at days 7 and 21 following nerve injury, while Kv1.4 and Kv1.6 were significantly upregulated at the neuroma site (Figure 1). We looked at Kv1 channel expression in the DRG using IHC and we observed that Kv1.2 expression is reduced after injury, while Kv1.4 and Kv1.6 expression remains unchanged (Figure 6a) (this is at the DRG soma, although expression could be seen to increase within paranodes/juxtaparanodes after injury). We also quantified protein expression within the DRG over the same time course using Western blot analysis. The expression of Kv1.2 was significantly reduced following nerve injury (Figure 6b–e) consistent with previous findings, (Everill et al., 1998; Ishikawa et al., 1999; Kim et al., 2002; Yang et al., 2004), and there was a trend for a reduction in Kv1.1 although this did not reach significance. The expression of Kv1.4 and 1.6 within the DRG did not significantly change following injury (one Way ANOVA, n = 6 per group) suggesting that increased expression within the juxtaparanode and paranode at the neuroma site is a likely consequence of altered trafficking of these proteins rather than global changes in expression.

We next looked into human nerve tissue to see if these changes were relevant to patients with NP We collected 6 control samples (from subjects having their sural nerves removed to use as a bridge for hand reconstructive surgery) and 6 samples obtained from patients undergoing removal of Morton's neuroma (interdigital nerve entrapment neuropathy). IHC (n = 3 per group, 8–10 nodes per patient) showed that only Kv1.2 is expressed in the juxtaparanode of healthy subjects (90 ± 10% of nodes were Kv1.2 positive) with absent Kv1.4 and 1.6 staining as observed in the rat. However, in neuroma Kv1.2 expression in the juxtaparanode was minimal (13.3 ± 8.1%) whilst Kv1.4 and Kv1.6 were expressed in most of the nodes (92.5 ± 7.4% for Kv1.4; 73.5 ± 8.8 for Kv1.6) (Figure 7a–b). We used western blotting to quantify Kv1 channels proteins in the nerves of the patients (n = 6 per group) and found that Kv1.2 expression was significantly decreased in neuroma compared to control nerve (to 0.48 ± 0.1 of the control, Mann-Whitney U-Test, p 0.005). In contrast, expression of Kv1.4 and Kv1.6 were significantly increased in neuroma compared to controls (to 6.3 ± 3.5, and 9.4 ± 6.6 of the control respectively, Mann-Whitney U-Test p = 0.005 both) (Figure 7c–d).

Figure 6.

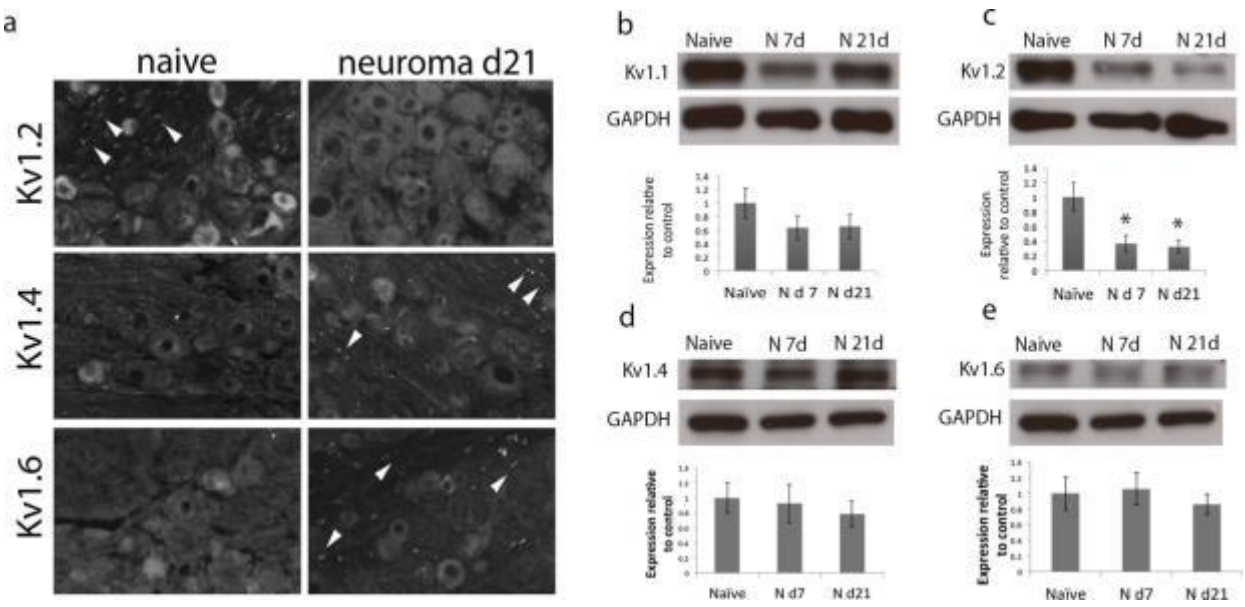
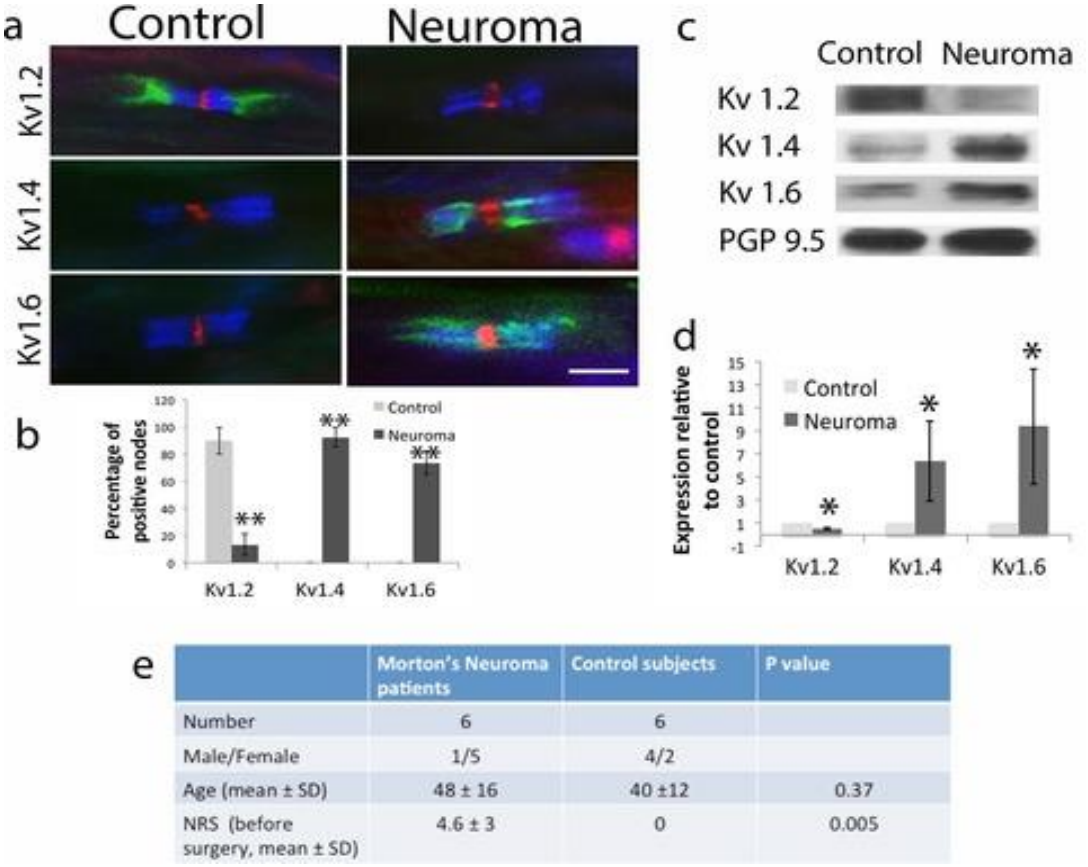


Figure 6. Expression of Kv1 channels in the DRG in the naıve state, 7 and 21 days after axotomy (neuroma). (a) Representative sections of naive and neuroma day 21 DRG immuno-stained with Kv1.2, Kv1.4, and Kv1.6. Note that Kv1.2 expression in DRG cells and axonal juxtaparanodes (arrow heads) is reduced after injury, while Kv1.4 and Kv1.6 expression remains unchanged in DRG cells, and it is present in axonal juxtaparanodes (arrow heads) after injury. In each panel (b–e), a representative blot is shown for each time point with GAPDH as a loading control. Quantification of 6 animals per condition is shown below (b, d, e) Expression of Kv1.1, Kv1.4 and Kv1.6 within the DRG does not significantly change after axotomy. (c) Kv1.2 expression is significantly decreased after axotomy. (*p<0.05, one Way ANOVA, n = 6 per group).

Figure 7.



e

	Morton's Neuroma patients	Control subjects	P value
Number	6	6	
Male/Female	1/5	4/2	
Age (mean ± SD)	48 ± 16	40 ±12	0.37
NRS (before surgery, mean ± SD)	4.6 ± 3	0	0.005

Figure 7. Kv1 channels expression in the sural nerve of healthy volunteers (control) and from patients with painful Morton neuroma.

(a) Representative sections of longitudinal nerves immunostained with Kv1 channels in green (Kv1.2, Kv1.4 and Kv1.6 respectively), a panNav antibody in red (to identify the node), and caspr in blue (to identify the paranode). Kv1.2 is expressed in the juxtaparanode in control nerves but it is not present in the injured nerve. Kv1.4 and kv1.6 are not present in control nerve but are expressed in neuroma within the juxtaparanode and encroaching on the paranode nodes. (b) Quantification of the percentage of Kv1.2, Kv1.4, and Kv1.6 positive nodes in control and neuroma nodes (n = 3 per group, one way ANOVA). (c) Western blots showing expression of Kv1 channels in control and neuroma nerve. (d) Quantification of WBs (n = 6 per group, one way ANOVA). Kv1.2 is expressed in the control nerve and down regulated after axotomy, while Kv1.4 and Kv1.6 have a low/null expression in the control nerve and are up-regulated in neuroma. PGP9.5 was used as a loading control. Error bars denote (e) Patients and control subjects demographic data. The female/male ratio is different in patients with Morton neuroma and controls (patients with traumatic lesion of the hand) reflecting the F/M ratio of these different conditions. The mean age of patients with Morton neuroma is slightly higher than in control subjects, although it is not significant (t-test). All patients with Morton neuroma presented with pain (mean NRS 4.6), while control presented no pain in the area innervated by the sural nerve (Mann Whitney test). NRS: numerical rate score. Error bars denote SEM. Scale bars = 5 μm, **p<0.001, *p<0.05.

Kv1 channels change their distribution in the nodal regions at sites distant from the injury.

We investigated the localisation of Kv1 channels at a site distant from the injury site. To do so, we used a model of L5 spinal nerve transection (SNT) (Kim and Chung, 1992) and studied the dorsal roots (ie. proximal to the DRG). We used this model instead of the neuroma model to have certainty that all the dorsal root axons studied had their peripheral terminals injured.

In the dorsal roots from naıve animals, the distance between Nav channels staining and the end of caspr was 3.5 ± 0.2 mm, and the distance between Nav channels staining and the start of Kv1.2 staining was 4 ± 0.2 mm, resulting in a small positive difference between these distances (0.5 ± 0.1 mm, n = 4 animals, 25–32 nodes per animal), indicating there was no overlap. Seven days after transection of L5 spinal nerve this distance was still positive (0.24 ± 0.05 mm, n = 4 animals; distance Nav-end caspr 3.7 ± 0.2 mm, distance Nav-start Kv1.2 3.9 ± 0.1 mm; 30–40 nodes per animal). However, at 21 days after nerve injury, we noted a significant overlap between the end of caspr staining and the start of the Kv1.2 staining (-1.4 ± 0.3 mm, n = 4 animals, 38–40 nodes per animal, one way ANOVA, $p<0.001$; distance between Nav-end caspr 3.9 ± 0.4 mm, distance between Nav-start Kv1.2 2.5 ± 0.4 mm). We observed this novel localisation of Kv1.2 in the paranode which (in contrast to the neuroma site) is still clearly present after nerve injury within the dorsal root. We also observed novel expression of Kv1.4 and 1.6 in the dorsal root of injured animals, and these were localised to the par- anode in addition to the juxtaparanode (the distance between end of caspr and start of Kv1.4 and 1.6 staining was -1.7 ± 0.6 mm and -1.07 ± 0.2 mm respectively; for Kv1.4 distance between Nav-end caspr 3.6 ± 0.4 mm, distance between Nav-start Kv1.4 1.9 ± 0.6 mm; for Kv1.6: distance between Nav-end caspr 3.4 ± 0.4 mm, distance between Nav-start Kv1.6 1.6 ± 0.4 mm n = 4 animals per group, 30–35 nodes per animal Figure 8).

Contactin-associated protein-like 2 (Caspr2) is normally localized at the juxtaparanode and associates with K+ channels (Chiu et al., 2014). Interestingly, we observed that caspr2 is mobilized into the paranode regions in a similar way to Kv1 channels; the difference between the Nav-caspr stain- ing distance and the Nav-caspr2 distance is minimal in naı̈ve roots (0.5 ± 0.1 mm; distance between Nav-end caspr 3.8 ± 0.3 mm, distance between Nav-start caspr2 4.3 ± 0.4 mm, 30–38 nodes per ani- mal 4 animals), and after SNT, this distance becomes negative (-0.88 ± 0.1 mm, n = 5 animals; dis- tance between Nav-end caspr 3.4 ± 0.3 mm, distance between Nav-start caspr2 2.5 ± 0.3 mm 35–40 nodes per animal $p<0.001$, t-test) indicating an overlap between caspr and caspr2 immuno-labeling (Figure 8).

Figure 8.

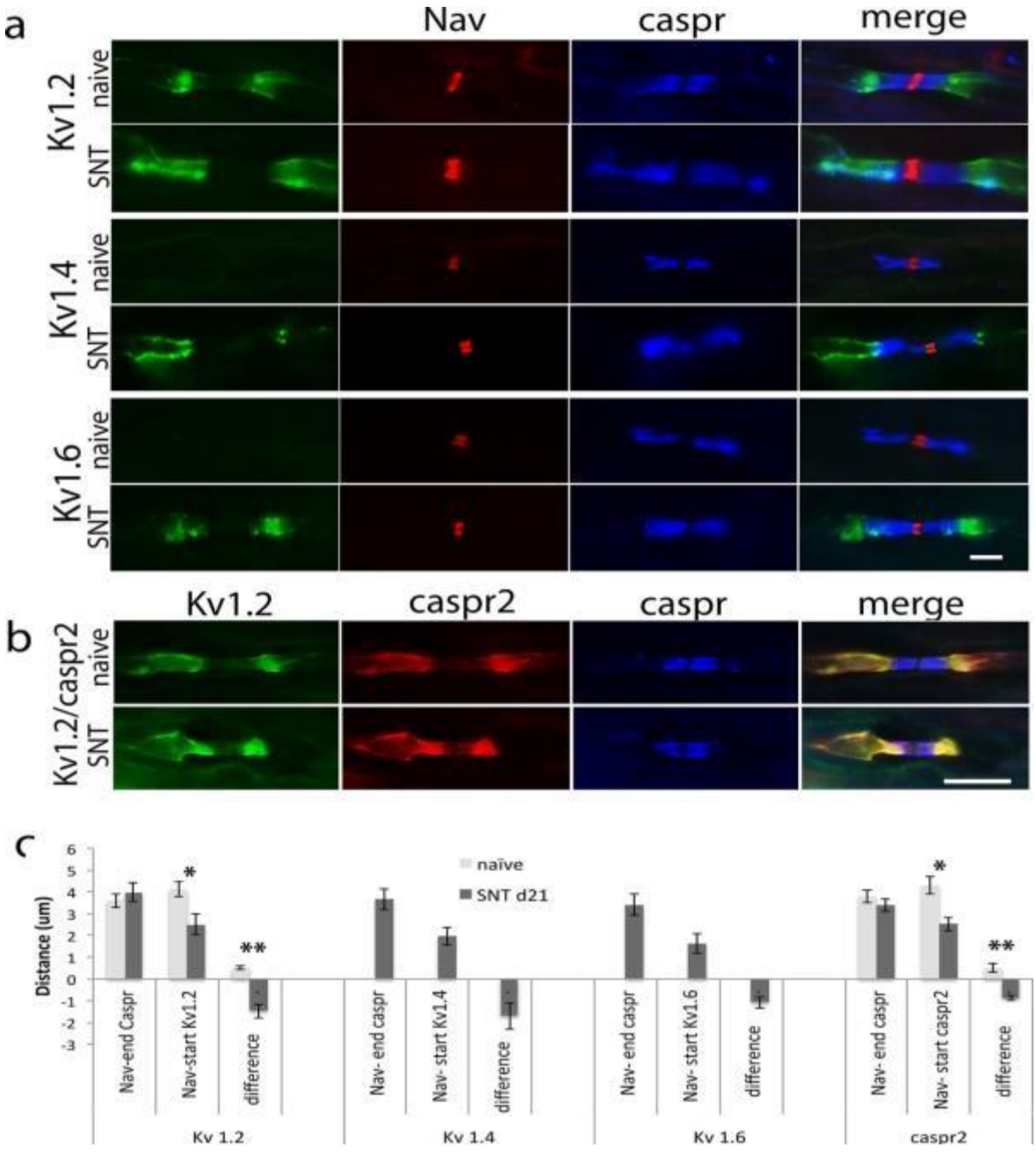


Figure 8. Kv1 channels expression in the dorsal roots of na ̈ive animals and 21 days after spinal nerve transection (SNT). (a) Representative sections of longitudinal dorsal roots immunostained with Kv1 channels in green (Kv1.2, Kv1.4 and Kv1.6, respectively), a panNav antibody in red (to identify the node), and caspr in blue (to identify the paranode). Kv1.2 is expressed only in the juxtaparanode in naı ̈ve nerves but after injury it invades the paranode. Kv1.4 and kv1.6 are not present in uninjured nerve but are expressed within the juxtaparanode after nerve injury and also invade the paranode. (b) Relocalization of caspr2 at 21 days after spinal nerve transection (SNT). Representative sections of longitudinal L5 dorsal roots immuno-stained with caspr2 in red, Kv1.2 in green, and caspr in blue. Kv1.2 and caspr2 are

expressed in the juxtaparanode in naı¨ve nerves but co-localized with caspr staining at 21 days after injury. (c) Quantification of: distance between the sodium channel staining (Nav) and the end of the caspr staining, distance between the sodium channel staining (Nav) and the start of the Kv1.2/1.4/1.6/caspr2 staining, and difference between these distances. A negative value in this difference represents an overlap of paranodal and juxtaparanodal proteins. Note that the distance between the sodium channel staining (Nav) and the end of the caspr staining remains unchanged after nerve injury, while the distance between the sodium channel staining (Nav) and the start of the Kv1.2/1.4/1.6/caspr2 staining is significantly shortened after nerve injury. Kv1.4 and Kv1.6 were absent in naive (n = 5 animals/4 sections per animal, *p<0.05, **p<0.001). Scale bars = 5 mm.

Effect of Kv1 channels redistribution and change in expression on the incidence of spontaneous activity

Spontaneous activity in naı¨ve axons was present in less than 5% of A-fibres (4.5%). We assessed the effect of blocking the Kv1 channels using a-DTX, a toxin isolated from black and green mamba snakes which is a selective and effective blocker of Kv1-containing oligomers composed of Kv1.1, Kv1.2, or Kv1.6 subunits (Harvey, 2001). We applied the toxin at the neuroma site (or, in control ani- mals, acutely cut sciatic nerve stump) and to the L5 DRG and recorded from sensory axons in thin strands dissected from the dorsal root. In the naı¨ve situation (n = 222 neurons, 10 animals, Figure 9a), the incidence of spontaneous activity did not significantly change after a-DTX (5.4 and 9.8% of myelinated afferents when the toxin was applied to nerve stump or L5 DRG, respectively). Two days after transecting the sciatic nerve (n = 291 neurons, 4 animals), spontaneous activity at the injured nerve increased to 22% of myelinated afferents, and it was similar with or without toxin (toxin applied to neuroma 26.1%, toxin applied to the L5 DRG 26%). However, at 7 days after nerve injury (n = 241 neurons, 7 animals) the proportion of spontaneously active afferents had decreased to 6.2%, and application of the toxin now induced a significant increase in proportion of spontaneously firing myelinated afferents (11.2% toxin at the neuroma p = 0.07, and 15.6% toxin to L5 DRG, p = 0.002, chi-square test). At day 21 after injury (n = 237 neurons, 7 animals) spontaneous activity has decreased to baseline levels (2.5% of afferents within the dorsal root), but application of the toxin to both neuroma site or L5 DRG significantly increased the proportion of myelinated afferents demonstrating spontaneous activity (7.2% p = 0.03 and 17.7 ±% p<0.001 respectively, chi-square test) (Figure 9a). In summary, we observed an acute increase in spontaneous activity following nerve injury, which was reversed with time. However, at this later time, blockade of Kv1 channels (which had no effect in the na¨ive state) could reinstate spontaneous activity almost to levels seen acutely after nerve injury. Therefore, Kv1 channels appear to have a role in the recovery from increased excitability following nerve injury (Figure 9b–d).

Effect of Kv1 channel redistribution and change in expression on mechanical sensitivity

Because Kv1 channels have been shown to have a crucial role in mechano-sensitivity (Hao et al., 2013), we tested their role in hypersensitivity following nerve injury. In the presence of a-DTX, significantly more neurons responded to mechanical stimulation using a 15g von Frey filament at the neuroma site at day 21 (with 4 g: pre 17.9% post 21.8%; with 8 g: pre 22.1% post 26%; with 15 g: pre 24.7%, post 30.5%, p = 0.006, chi-square test; n = 259 neurons; Figure 9e–f).

Figure 9.

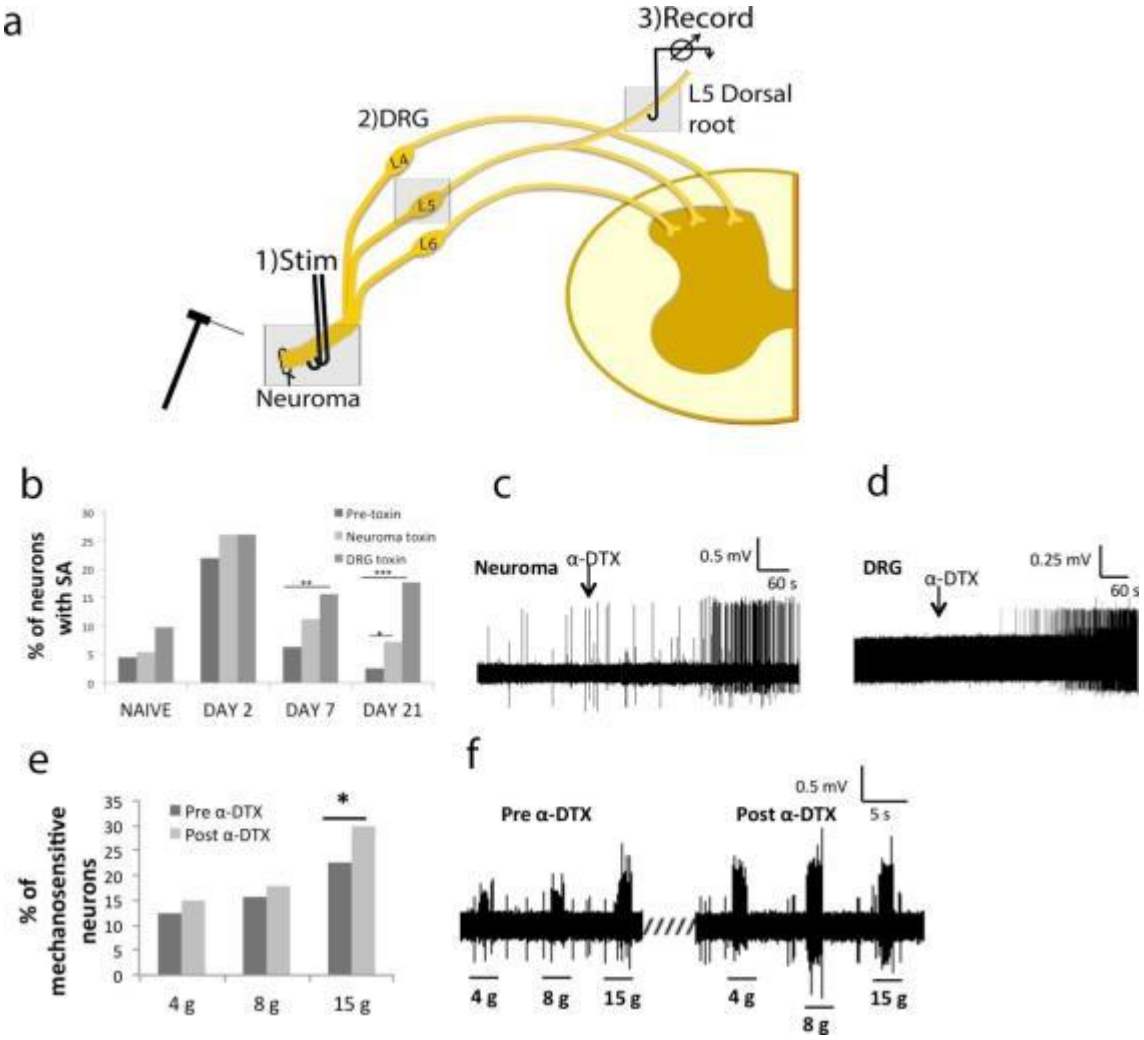


Figure 9. Local application of a-DTX reinstates primary afferent hyperexcitability at later time points following nerve injury. (a) Schematic illustration of 3-chamber recording system. 1) Recording chamber, 2) middle chamber, 3) stimulating chamber. The toxin was applied in chambers 1 or 2, respectively. (b) Following sciatic nerve transection, there is a large increase in the proportion of primary afferents demonstrating spontaneous activity at day 2, which is suppressed at days 7 and 21 post injury; Local application of a-DTX to the neuroma and L5 DRG at these later time points (days 7 and 21) significantly increases the proportion of afferents, which are spontaneously active (total proportions per group, chi-square tests, *p<0.05, **p<0.01, ***p<0.001) (c) neuroma application and (d) DRG application of a-DTX. Both recordings were carried out 21 days post-surgery. (e) In the presence of a- DTX, significantly more neurons respond to mechanical stimulation at the neuroma site using a 15 g von Frey filament (*p<0.05; total proportions per group, chi-square tests, all neuroma day 21). (f) Representative traces showing greater responsiveness to mechanical stimulation with von Frey filaments after local a-DTX application.

Kv1 channels are responsible for the partial recovery of mechanical hypersensitivity seen chronically after nerve injury

We subsequently used behavioural measures to examine mechanical sensitivity after nerve injury. For this purpose, we used the sciatic neuroma model used before in which the sciatic nerve of rats is transacted and the proximal end was sutured superficially below the skin on the animal's leg. We applied von Frey filaments of increasing forces to the site of the skin covering the neuroma. To test the role of Kv1 channels on hypersensitivity, we applied a DTX subcutaneously at the site of neuroma. At baseline, the withdrawal threshold was high and did not change with the application of a DTX (vehicle: 142.9 ± 13 g, toxin 150 ± 17.7 g, n = 9 animals per group). Three days after injury, this threshold dropped to 8.2 ± 0.6 g and was not changed by applying the toxin (9.7 ± 1.1 g) (n = 9 animals per group). However, with time this threshold began to normalise reaching 12.9 ± 0.6 g at 7 days (n = 8) and 44.8 ± 1.6 g at 21 days (n = 8). When we injected aDTX, this recovery was not seen and thresholds stayed low (day 7: 6.9 ± 0.9 g, p = 0.003, n = 9; day 21: 13 ± 2.1 g, p<0.001, n = 7, RM two way ANOVA Figure 10).

The time point when the mechanical hypersensitivity began to recover in injured animals coincides with the time when Kv1.1 and Kv1.2 are reduced but Kv1.4 and Kv1.6 are being expressed. a-DTX is a selective blocker for Kv1.1, Kv1.2 and Kv1.6 but has little activity against Kv1.4. CP 339818 hydro- chloride however, which is a selective blocker of Kv1.3 and Kv1.4 (Nguyen et al., 1996), did not have any effect on mechanical hypersensitivity at early or late time-points post injury suggesting that Kv1.4 is dispensable for the suppression of hyperexcitability. (baselines: saline 133 ± 21 g, CP339818 164 ± 38 g; neuroma day 3: saline 51 ± 2 g, CP339818 44 ± 5 g; neuroma day 7: saline 41 ± 3 g, CP339818 39 ± 4 g; neuroma day 21: 73 ± 13 g, CP339818 91 ± 4 g, RM two way ANOVA, p>0.05, n = 8–7 for saline at day 21]).

Figure 10.

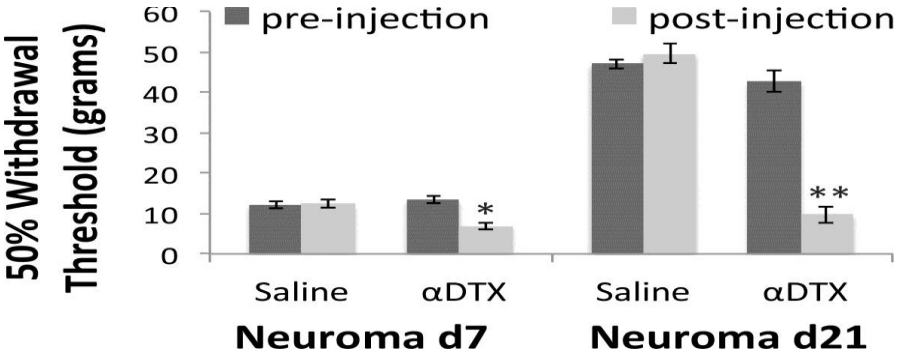


Figure 10. Mechanical hypersensitivity is restored by blocking Kv1 channels. Mechanical withdrawal thresholds were assessed by applying a range of Von Frey hairs to the skin over the neuroma site (labelled with a suture). Animals were randomised to receive either subcutaneous aDTX or saline 30 min before testing. Hypersensitivity after nerve injury is very pronounced until day 7, when it slowly starts recovering. At this time point, perineuromal application of aDTX reversed this early recovery. At 3 weeks after nerve injury hypersensitivity is much recovered and perineuromal injection of aDTX restored mechanical hypersensitivity to levels seen acutely after injury (RM two way ANOVA, *p<0.05, **p<0.001).

4. Discussion

We have focussed on the distribution of Kv1 channels within the axolemma of myelinated axons in rodent models of NP as well as in human neuroma tissue. We show that both at the site of injury (using the neuroma model) and within the dorsal root (remote from the injury site) that there is a major change in the a-subunit composition of Kv1 channels (with increased expression of Kv1.4 and 1.6) and furthermore that Kv1 channels are no longer restricted to the juxtaparanode but also re-distribute to the paranode following injury. Blockade of these ion channels using a-DTX reveals that following axonal injury Kv1 channels act to suppress axonal hyperexcitability and hence hypersensitivity to sensory stimuli (Figure 11).

Figure 11.

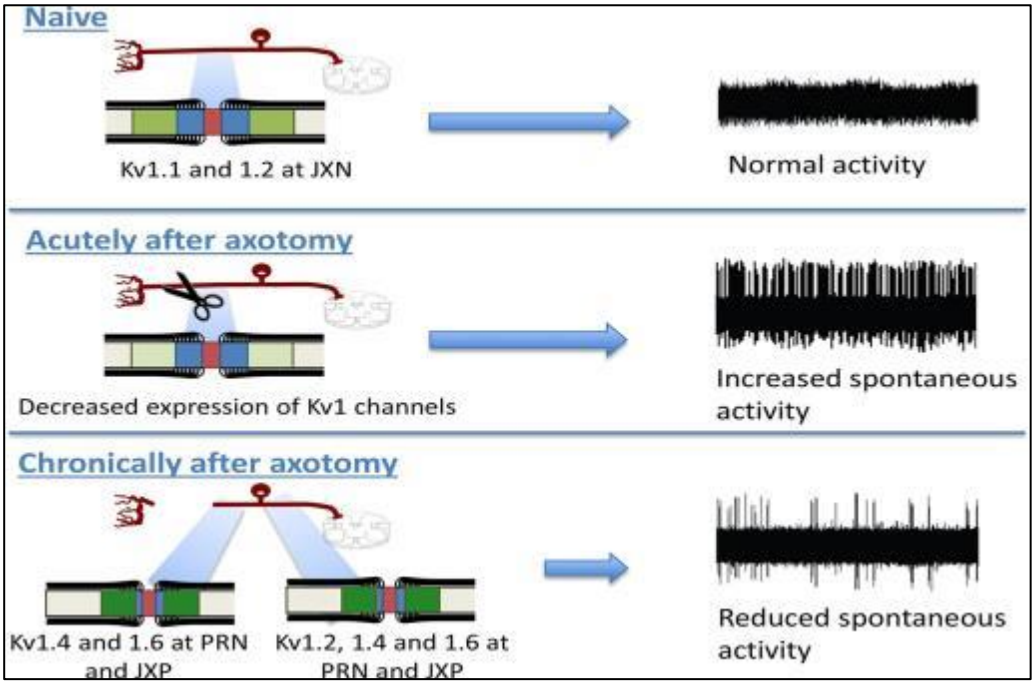


Figure 11. Schematic illustration of the changes in Kv1 channels subunit composition and distribution within the nodal complex and the relationship to hyperexcitability. In the na¨ive state, Kv1 channels (Kv1.1 and 1.2 shown in green) are localised to the juxtaparanode and separated from voltage-gated sodium channels at the node (red) by the paranode (blue). Acutely following axotomy myelinated primary afferents show a marked increase in spontaneous activity as a consequence of complex changes in increased pro-excitatory drive (for instance from voltage-gated sodium channels) as well as reduced 'brakes' on excitability At later stages within the neuroma, although there is less expression of Kv1.1 and Kv1.2, the expression of Kv1.4 and 1.6 increases. Remote from the injury within the dorsal root expression of Kv1.1 and 1.2 is maintained and the expression of Kv1.4 and Kv1.4 also increases. Furthermore Kv1 channels are redistributed to the paranode as well as being expressed within the juxtaparanode. These changes are associated with a suppression of hyperexcitability.

We initially examined the distribution of Kv1 channels within the juxtaparanode of axons within the neuroma at the the injury site. Following traumatic nerve injury there is an initial inflammatory response, demyelination and axonal dieback within the proximal nerve stump. This is followed by axonal sprouting (Fried and Devor, 1988; DeFelipe, 1991) (which is often misdirected) as well as proliferation of connective tissue and glial cells. This can lead to a region of nerve swelling, a neuroma, the development of which is well documented in patients (Scadding, 1981; DeFelipe, 1991; Young, 1942). Nerve injury is often associated with ongoing pain, dysaesthesia and evoked pain; many patients report that touching the skin overlying or compression of the neuroma site can elicit pain and dysaesthesia (Nikolajsen et al., 2010). Unfortunately in many patients, the treatment of NP remains inadequate (Finnerup et al., 2015) and is associated with significant dis- ability (Stokvis et al., 2010). Neuroma pain can be modelled in the rodent using a modified version of the tibial transposition neuroma model (Dorsi et al., 2008).

Altered distribution of Kv1 channels in nodal complexes of the neuroma and dorsal root

It has previously been documented that within a neuroma nodes of Ranvier become disorganised (Levinson et al., 2012) and we confirm that here with the majority nodes showing abnormalities such as being elongated, split, heminodes or showing Na_V in the absence of caspr. Voltage-gated sodium channels are known to accumulate particularly within axon tips and on denuded axons of the neuroma (England et al., 1996). Much less is known regarding the distribution of Kv1 channels changes within the neuroma. This is an important issue as such channels could potentially act as 'brakes' on excitability. At an early time point after injury, we found that Kv1.2 is no longer confined to the juxtaparanode but extended into the paranode. As this could be only a reflection of direct injury, we also looked into a site in the injured nerve 1 cm proximal to the neuroma and found similar changes in distribution, suggesting this is likely to reflect widespread changes within the axon/axoglial signalling (further supported by changes within the dorsal root and discussed below). At a later time-point following injury, we found a striking reduction in the expression of Kv1.1 and 1.2 which are normally localised to the juxtaparanode. In contrast Kv1.4 and 1.6 which are present at a low level in the naıve state are up-regulated and are present both in the paranode and the juxtaparanode. We found broadly similar changes in rodent and human neuroma. This altered expression and localisation is likely to partially reflect the altered relationship between axons and myelinating Schwann cells. Within the neuroma new nodes of Ranvier will be formed as a consequence of myelination of new axon sprouts and remyelination of denuded axons (Dorsi et al., 2008; Dyck et al., 1985). During developmental myelination and remyelination following primary demyelination (in which the axon remains intact) Kv1.1 and 1.2 can at early time points be observed in other regions apart from the juxtaparanode (at the node of Ranvier and the paranode) before being restricted to the juxtaparanode as the nodal complex matures (Rasband et al., 1998; Vabnick et al., 1999; Poliak et al., 2001). Kv1.4 and 1.6 expression has not to our knowledge been examined during myelination/remyelination.

We also studied the dorsal root, which is remote from the injury site to establish whether there were changes in Kv1 channels composition of the juxtaparanode that reflect the general response of the axon to injury rather than local effects such as inflammation and remyelination at the injury site. We also found striking changes within the nodal complex of sensory axons within the dorsal root. Kv1.2 was no longer down regulated as had been noted at the neuroma site but its localisation changed following injury: They could be observed in the paranode as well as the juxtaparanode. In the naıve state, very little Kv1.4 and

1.6 expression was noted in the juxtaparanode of the dorsal roots. This is in agreement with previous studies that reported a low frequency of Kv1.4 immunoreactive juxtaparanodes (Everill et al., 1998) and no expression of Kv1.6 (Utsunomiya et al., 2008). We found however that nerve transection led to markedly increased expression of these a-subunits and they were localised both to juxtaparanode and paranode.

Factors governing localisation of Kv1 channels to axonal domains in the naıve and injured state

What factors are responsible for the altered distribution of Kv1 channels within the juxtaparanode? We used ultrastructural examination of the juxtaparanode in the dorsal root to examine whether structural changes within the paranode could explain the movement of Kv1 channels into this region (from which they are normally excluded). The transverse bands are important points of attachment between the axon and the paranodal loops of the Schwann cell. These axoglial septate-like junctions are formed by the interaction of caspr (Bhat et al., 2001) and contactin (Boyle et al., 2001) on the axolemma binding with the 155Kd isoform of Neurofascin expressed on the Schwann cell paranodal loops (Tait et al., 2000). These junctions act as diffusion barriers between the nodal and juxtaparanodal membrane. Mice lacking caspr (Bhat et al., 2001), contactin (Boyle et al., 2001) or NF155 (Pillai et al., 2009; Sherman et al., 2005) have absent transverse bands, increased distance between the axon and Schwann cell membrane, disorganisation of the paranodal loops and probably as a consequence of the loss of this lateral diffusion barrier Kv1 channels are noted to extend into the paranode. Similarly in mice lacking ceramide galactosyl transferase in which all-putative adhesion components of the paranodal junction are lacking, Kv1.2 and caspr2 are also mislocalised to the paranodes (Poliak et al., 2001; 2003). On ultrastructural examination of the paranodes within the sciatic nerve following injury, we did not see major structural changes and there was no increase in the distance between the axon and the Schwann cell membrane at the site of attachment of the paranodal loops. We noted an increase in the maximum distance between paranodal loops, which is unlikely to alter the ability of molecules to passively diffuse between the membrane domains of the juxtaparanode and paranode (however it will increase the diameter of the helical pathway between paranodal loops connecting the extracellular space to the axonal internode. One potential consequence of which would be reduced passive resistance to current flow between the node and voltage-gated potassium channel (VGKC) in the juxtaparanode and paranode, which could then have a greater influence on nodal excitability (Shroff et al., 2011). A recent publication has demonstrated that in mice lacking bII spectrin expression in axons Kv1 channels were no longer restricted to the juxtaparanode but could also be observed in the paranode even though the structural integrity of axoglial junctions was intact (Zhang et al., 2013). bII spectrin contributes to the sub-membranous cytoskeleton of the axon-linking membrane proteins to actin and appears to act as a barrier limiting the lateral diffusion of membrane proteins. We found that paranodal expression of bII spectrin was reduced following axotomy and a reduction in this sub-membranous barrier is compatible with the lateral movement of Kv1 channels into this region that we observed. As well as Kv1 channels we also see caspr2 overlapping with paranodal markers following nerve injury and again such paranodal localisation of caspr2 was also reported in mice in which axonal bII spectrin is conditionally ablated. Caspr2 complexes with and is important for the correct localisation of Kv1 channels (Poliak et al., 1999; 2003) suggesting that this whole protein complex is mislocalised following nerve injury. Although loss of a sub-membranous barrier to diffusion of the VGKC-complex is one explanation for their paranodal localisation, we do not yet have a full understanding of the regulation of Kv1 channels trafficking. Phosphorylation events may have a role to play as Kv1.2 can

undergo phosphorylation, which impacts on surface expression/localisation (Gu and Gu, 2011; Yang et al., 2007).

The effect of altered Kv1 channels subunit composition and localisation on axonal excitability and NP

Following axonal injury sensory axons become hyper-excitable and this is important in driving and maintaining NP (Han et al., 2000). A recent study showed that myelinated sensory fibres are key in maintaining mechanical allodynia in several NP models (Xu et al., 2015). Spontaneous activity and mechanical stimulus evoked activity has been recorded in myelinated afferents innervating neuroma using microneurography (Nystro¨m and Hagbarth, 1981). The role of Kv1 channels has mainly focussed on their importance in suppressing excitability at the soma rather than the axon following injury. The expression of a number of a Kv1 channels sub-units has been documented to decrease following peripheral axotomy including Kv1.1, 1.2 (Everill et al., 1998; Hao et al., 2013; Ishikawa et al., 1999; Kim et al., 2002; Yang et al., 2004) and in some reports Kv1.4 (we did not see a reduction in Kv1.4 using western blot analysis of DRG lysate following sciatic axotomy, however, this is a less proximal lesion compared to spinal nerve ligation [Everill et al., 1998]). Correspondingly, the K currents mediated by such channels are reduced when measured at the soma (Yang et al., 2004) both in small and large diameter DRG cells. The focus has therefore been on the loss of K currents within the soma which normally act as a 'break' on excitability, and combined with increased excitatory drive for instance due to the dysfunction of voltage- gated sodium channels and hyperpolarization-activated cyclic nucleotide-gated (HCN) channels this leads to ectopic activity (Waxman and Zamponi, 2014). While changes at the soma are undoubtedly important, ectopic impulses also arise along the axon. There has been much less focus on the distribution and function of Kv1 channels within the axon following peripheral nerve injury.

The function of Kv1 channels is critically dependent on their targeting to specific neuronal compartments (Trimmer, 2015). The expression of Kv1 channels within the DRG soma and the axon should not be assumed to be the same: For instance the expression of Kv1.6 remains stable within the DRG both at the level of mRNA and protein (see Kim et al. (2002), Yang et al. (2004) and our own data) but as we show here expression markedly increases in axons within peripheral nerve and dorsal root. Altered a subunit composition and localisation of Kv1 channels is likely to have functional implications. In the naıve state, the Kv1 channels Kv1.1 and 1.2 are located within the juxtaparanode, below the insulating myelin sheath and at least in peripheral nerves have little functional impact on nodal excitability and conduction (Poliak et al., 2003; Chiu and Ritchie, 1980; Sherratt et al., 1980; Rasband et al., 1998). During development when Kv1 channels are observed in the node and paranode using specific blockers of this K current suggests that these Kv1 channels prevent re-entrant excitation in motor axons (Vabnick et al., 1999). Following primary demyelination, the redistribution of Kv1 channels to the paranode acts to suppress continuous conduction in demyelinated axons (Rasband et al., 1998). In certain contexts therefore and especially when Kv1 channels begin to encroach on the paranodal regions there is evidence that these channels can sup- press excitability of the axon. As has been previously demonstrated we have found that the rate of ectopic activity within myelinated axons is very high in the first week and then decreases the longer the time elapsed following the initial injury (Campbell et al., 1988). Understanding adaptive mechanisms to suppress such hyper-excitability will potentially provide insight as to why in certain patients such mechanisms fail leading to chronic pain states. Over a similar time period as this reduction in spontaneous activity we have observed increased expression of Kv1.4 and 1.6 as well as redistribution of Kv1 channels to the paranode and juxtaparanode domains. Selective inhibition of Kv1 channels with a-DTX reinstates a

higher level of ectopic activity, increases mechanosensitivity of afferents innervating the neuroma and on behavioural testing also exacerbates mechanical hypersensitivity, which had begun to normalise at 3 weeks post injury. a-DTX blocks Kv1.1, Kv1.2 and Kv1.6. As expression of Kv1.1 and 1.2 are decreased while Kv1.6 is increased, most probably the effect seen with the toxin is through Kv1.6. Selective inhibition of Kv1.4 did not recapitulate these events emphasising the role of Kv1.6 (and subunits with which it complexes) in suppressing hyperexcitability. Neuronal Kv1 proteins form heterotetramerization of a subunits, which also associate with auxiliary Kv b subunits (Jan and Jan, 2012), adding complexity in ascribing function to individual a subunits. a subunits confer particular pharmacological and biophysical properties on these channels and in addition there may be interactions between subunits. For instance Kv1.4, usually shows N- type rapid inactivation through an N-terminal inactivation ball however this can be over-ridden if associated with a Kv1.6 a subunit (Roeper et al., 1998), due to its NIP (N-type inactivation prevention) domain.

5. Conclusion

In conclusion, we have found major changes in Kv1 channels subunit composition and distribution within the axolemma of myelinated axons following traumatic nerve injury. In contrast to the soma in which Kv1 channels expression is reduced this increased availability of Kv1 channels within the paranodes and altered subunit composition appears to fulfil an adaptive role in suppressing excessive excitability in myelinated afferents.

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Chapter 2:

Symptom profiles in the painDETECT questionnaire in patients with peripheral neuropathic pain stratified according to sensory loss in quantitative sensory testing

1. Introduction

Neuropathic pain (NP) is defined as pain resulting from a lesion or disease of the somatosensory system (Treede et al. 2008, Baron et al. 2010) and can be caused by many clinical etiological entities such as nerve injury, herpes zoster or polyneuropathy (Baron et al. 2010, Maier et al. 2010). Identification of probable NP is based on a plausible medical history with the distribution of pain consistent with the innervation territory of the suspected lesioned nerve structure. Further, a clinical examination including the assessment of negative (loss- of-function) or positive (gain-of-function) sensory signs is required. The sensory phenotype can be assessed via bedside testing (Leffler and Hansson 2008) or Quantitative Sensory Testing (QST) (Krumova et al. 2012a, Drangholt et al. 2013). The underlying lesion or disease of the somatosensory system can then be further examined by a diagnostic test, e.g. electroneurography, skin biopsy or corneal confocal microscopy (CCM) (Lacomis 2002, Treede et al. 2008, Haanpää et al. 2011). Loss of sensation implies a dysfunction, lesion or disease in different types of nerve fibers: loss of function of the thickly myelinated Aβ-fibers can be demonstrated by clinical testing of the sensation of touch and vibration (Leffler and Hansson 2008) or tactile and vibration detection threshold (e. g. in QST). Loss of small fiber (Aδ- and C-fiber) function is characterized by hypoesthesia to thermal stimuli, which can be tested by using QST by assessing the cold and warm detection threshold or in bedside testing by cold and warm metallic rollers or water tubes and pins (Lacomis 2002, Lauria et al. 2012). Both types of sensory loss may appear in patients suffering from NP separately, in combination or might not appear at all. It has been shown that they may be important predictors for the response of patients to certain medications (Demant et al. 2014, Mainka et al. 2016). For use in primary health care practice, a simpler tool, such as a questionnaire that could identify patients with core symptoms of neuropathic pain would be valuable.

The painDETECT Questionnaire (PDQ) was developed and validated to support the identification of neuropathic pain components in patients suffering from chronic pain of different origin (Freynhagen et al. 2006). It has previously been shown that pain descriptors of the PDQ correlate with QST items testing related pain thresholds in patients suffering from radiculopathy, but not in patients suffering from fibromyalgia (Tampin et al. 2013). We undertook this exploratory analysis in a larger sample prospective study because the PDQ was developed before a clear definition of NP was established (Treede et al. 2008). Therefore, it was never part of the validation process of PDQ to investigate whether different types of sensory loss (as described above) present with different PDQ profiles or if single PDQ items are sensitive to types of sensory loss in a clinical examination. This would be useful to validate the PDQ not only as a screening tool for NP itself, but also for different sensory subtypes of NP. The aim of the study was to analyze if the overall PDQ score or its items reflect phenotypes of sensory loss in NP as determined by QST.

Within the European consortia IMI (Innovative Medicines Initiative) Europain and Neuropain, both QST data and PDQ results of patients with neuropathic pain assessed by pain research units across Europe were gathered in a central database, enabling an analysis of the correlation between QST obtained somato-sensory profiles of thermal and/or mechanical loss of function and PDQ profiles.

2. Methods

Patient cohort

The joint European database of the IMI-Europain and Neuropain consortia, two prospectively collected cohorts using identical study protocols conducted in parallel, contains 580 data records of patients with lesions or diseases of the somatosensory nervous system. The recruitment of these two prospective studies started in March 2011 and the database was merged and finally closed in December 2013. Both studies were approved by local ethics committees according to local regulations. All subjects provided a signed written informed consent according to the current version of the Declaration of Helsinki for participation in the respective study and transfer of the study records into the central database. Only patients with complete QST and PDQ (unless in the case of a painless neuropathy) were included into the database. All centers and investigators underwent a strict quality assessment and certification process to ensure that we were able to pool future data across sites and countries (Magerl et al. 2010, Vollert et al. 2015). A confirmatory analysis of heterogeneity between the participating centers in healthy subjects and patients with painful polyneuropathy or peripheral nerve injury showed a high degree of homogeneity among the different centers, making it possible to analyze the database as a homogenous group (Vollert et al. 2016). With only few exceptions, patients performed QST and PDQ on the same day. In case of deviation from the same day assessment, the PDQ was completed within a week after QST. QST and PainDETECT both are reported to be highly reliable to normal test-retest fluctuation within a few days (Geber et al. 2011, Keller et al. 2016).

The main inclusion criterion for this analysis was that all patients in the central database had pain for more than 6 months, with a mean pain intensity in the last four weeks of ≥ 2 on a Numerical Rating Scale, NRS, 0 – 10 and pain due to polyneuropathy (PNP), peripheral nerve injury, postherpetic neuralgia, radiculopathy or trigeminal neuropathy (n = 336). As we were aiming to specifically explore commonalities across neuropathic pain entities and to obtain as generalizable information as possible, diagnoses were pooled and not analyzed separately.

Europain Consortium

The EUROPAIN project (http://www.imieuropain.org) was founded in 2009 and aims to improve the treatment of patients with long-term pain and consists of academic study groups working on pain research from Germany, Denmark and the UK. A Spanish SME (small and medium sized enterprises) and Europe's most active pharmaceutical companies working on pain also contributed. Data for this study was collected by the following centers: Ruhr-University Bochum, Germany, University of Schleswig Holstein, Kiel, Germany, Technische Universität München, Munich, Germany and Aarhus University, Denmark. The ethics committee of each center approved the study protocol separately.

Neuropain Consortium

The NEUROPAIN project is an investigator-initiated study consisting of several researchers in the field of Neuropathic Pain research within Europe (principal investigator: RB) and aims to characterize subgroups of patients with NP. Data for this study were collected by the following centers: Ruhr- University Bochum,

Germany, University of Schleswig Holstein, Kiel, Germany, Technische Universität München, Germany, Aarhus University, Denmark, Université Versailles-Saint-Quentin, Versailles, France, Helsinki University Central Hospital, Finland, Karolinska Institutet, Stockholm, Sweden, Benedictus Hospital Tutzing, Germany, Imperial College, London, UK, Heidelberg University, Germany, Neuroscience Technologies, Ltd., Barcelona, Spain. The ethics committee of each center approved the study protocol individually.

Central database

Each study centre used the computer-assisted program Neuroquast© (Statconsult, Magdeburg, Germany) for data entry locally. Study records were implemented into the central database on a monthly basis.

Investigations

Standardized patient assessments were performed by all the pain centers using the same case report forms (CRF).

Inclusion / Exclusion Criteria

For inclusion of patients in the central database, the diagnosis of underlying etiology and classification of pain as neuropathic was made by an experienced physician with a qualification in pain medicine and documented in the local center. Inclusion criteria for each diagnosis were as following:

- polyneuropathy: pathological electroneurography or pathologically decreased vibration detection thresholds at two of four sites (< 5/8) at the lower limb (Hilz et al. 1998, Martin et al. 2010), which could not be explained by another disease, or pain with polyneuropathy-type location and evidence of small fiber neuropathy based on skin punch biopsy, laser-evoked potentials or bedside thermal testing, which could not be explained by another disease.
- peripheral nerve injury: history of traumatic nerve injury of the distal upper or lower limb and sensory-motor abnormalities confined to the innervation territory of the injured nervous structure.
- postherpetic neuralgia: unilateral zoster rash in the facial or thoracic area with post-zoster scarring, hypo- or hyperpigmentation in the affected dermatome or sensory deficit in the area of the previous zoster rash determined by bedside-testing.
- radiculopathy: pain in the L5 and/or S1 dermatome and positive straight leg raising test or sensory deficit within the matching dermatome or diminished Achilles tendon reflex for S1 lesions and MRI of the lumbar spine confirming nerve root impairment by a herniated intervertebral disk or electromyography showing denervation in the L5 or S1 territory.
- trigeminal neuropathy: idiopathic sensory trigeminal neuropathy or iatrogenic mandibular neuropathy (i.e., inferior alveolar or lingual nerve neuropathy after various kinds of intraoral procedures) or trigeminal neuropathy secondary to compression, trigeminal neuropathy secondary to percutaneous lesions of the Gasserian ganglion and sensory loss in the neuroanatomical adequate trigeminal territory.

Exclusion criteria for entry in the database were age < 18 years, missing informed consent, insufficient language skills or other communication problems, pain treatment by topical local anesthetics for ≥ 7 days in the last 4 months or by topical capsaicin in the last 6 months, comorbidities treated by anticonvulsants or antidepressants, other pain locations with pain intensities ≥ 6 on ≥ 15 days/ month, other severe systemic or focal diseases of the central nervous system (e.g., stroke, spinal cord lesion), spinal canal stenosis, peripheral vascular disease (Fontaine stage II or higher), pending litigation and major cognitive or psychiatric disorders. In the cases of unilateral NP syndromes, patients with contralateral neuropathy or painful conditions of the contralateral limb were excluded. Datasets were excluded in the case of incomplete records (e.g., no precise diagnosis available, more than 2 missing variables of the QST in the affected area, no information about age, gender or other demographic data). painDETECT Questionnaire

The PDQ is a validated screening tool developed to aid in identifying NP and NP components (Freynhagen et al. 2006, Cappelleri et al. 2014). It comprises 9 items regarding the severity, course, quality and nature of the patient's pain. It screens for symptoms like burning, tingling or prickling sensations, pain evoked by light touch, thermal stimuli or light pressure, spontaneous pain attacks and numbness, which are linked to neuropathic components of the pain. Patients can rate the intensity of symptoms on a 6 point-Likert-scale from "never" to "very strongly". For the present analysis, the seven questions for the grading of sensory symptoms were used (Freynhagen et al. 2006).

Quantitative sensory testing

In accordance to the DFNS protocol, QST comprises a standardized battery of 13 different thermal and mechanical parameters and assesses both afferent small fiber (un- and thinly myelinated C and Aδ fibers, respectively) and large fiber function of the thickly myelinated Aß-fibers (Rolke et al. 2006, Krumova, Geber, Westermann, and Maier 2012b). The following parameters are part of the protocol: cold and warm detection threshold (CDT and WDT), alternating warm and cold stimuli (TSL: thermal sensory limen) during which the number of paradoxical heat sensations (PHS) is counted, cold and heat pain thresholds (CPT and HPT), mechanical detection threshold (MDT), vibration detection threshold (VDT), mechanical pain threshold for pinprick (MPT), pressure pain threshold for blunt pressure (PPT), mechanical (pinprick) pain sensitivity (MPS), dynamic mechanical allodynia for brush, cotton wool and Q-tips (DMA) and pain sensation to repetitive pinprick stimuli in the so-called wind up ratio (WUR).

Z-transformation of QST data

After Z-transformation QST values can be directly compared between patients, across different testing areas, age decades and gender. All QST values were z-transformed separately for each parameter (with the exception of PHS and DMA) (Rolke et al. 2006, Magerl et al. 2010, Pfau et al. 2014). Z-values above 1.96 indicate an individual level abnormal gain of function where the patient is significantly more sensitive to the tested stimuli compared to healthy controls of the same gender, of comparable age and tested in the same area (hyperesthesia, hyperalgesia). Z-scores below -1.96 indicate an individual level abnormal loss of function referring to a significantly lower sensitivity of the patient (hypoesthesia, hypoalgesia). A z-value of 0 indicates the mean of the healthy control group matched for age, gender and testing site; all values above 0 indicate gain of function, all values below 0 loss of function. This procedure leads to sensory

profiles of groups of patients that can be graphically displayed on one common scale for sensory loss and gain.

Subgrouping of Patients

Based on their QST profile, all data sets were sub-grouped according to the type of sensory loss, partial or total:

- i) patients with no abnormal loss of sensation in thermal and mechanical detection parameters,
- ii) patients with isolated abnormal loss of thermal sensation (loss of detection in the CDT and/ or WDT, representing pure loss of small fiber function),
- iii) patients with isolated abnormal loss of mechanical sensation (loss of detection in MDT and/ or VDT, representing pure loss of large fiber function),

iv) patients with both abnormal loss of thermal and mechanical sensation (representing loss of small and large fiber function).

Statistics

The IBM Statistical Package for the Social Sciences (SPSS) Version 22 was used for all statistical analyses. We analyzed each PDQ item for significant fiber effects in a two-factor analysis of variance (ANOVA), where each factor represents either the function of small or large fibers. Factor one was an abnormal loss of thermal sensation (sub-groups ii and iv, loss of small fiber function), factor two was an abnormal loss of mechanical sensation (iii and iv, loss of large fiber function).

3. Results

Characteristics of patients

Neuropathic pain in this combined patient sample from three consortia was most frequently caused by polyneuropathy (49%), peripheral nerve injury (24%) and radiculopathy (18%) (table 1). Across the cohort and the different etiologies gender was almost equally distributed, the average age was 58 (±15) years.

QST subgrouping

According to the cutoff of z-values below -1.96 (95% confidence interval of values found in a healthy control group matched in age and gender, assessed in the same area), 79 patients had no sensory loss (Fig. 1a), 55 only thermal sensory loss (Fig. 1b, CDT and WDT), 69 only mechanical sensory loss (Fig. 1c, MDT, VDT), and 133 a combination of the two (Fig. 1d). Mean z-scores in the groups with significant sensory loss according to the cutoff were lower than -1.96. In 86 cases (46%), if a patient had loss in either CDT or WDT, the same was true for the other quality. For mechanical detection thresholds, this was similar, though slightly less pronounced (78 cases (39%)). Patients without individually diagnosed sensory loss nonetheless had slightly negative mean z-scores (around -1) for detection thresholds and slightly positive mean z-scores for pain measures; this group also had the most pronounced pinprick hyperalgesia and dynamic mechanical allodynia. Patients with loss of thermal sensation (subgroups ii and iv) also had a loss for heat pain sensitivity (HPT, mean value for loss of thermal sensation = -0.98, $p < 0.001$, mean HPT value for loss of both thermal and mechanical sensation = -1.25, $p < 0.001$). Patients with loss of mechanical sensation (subgroups iii and iv) also had minor loss of thermal sensation with mean z-values around -1.00 in CDT and WDT.

Pain intensity did not differ between groups of sensory loss, neither current nor mean or maximum pain of the last four weeks. There were no significant differences in the frequency of intake of medication (table 2).

Table 1.

Disease	Polyneuropahy	Peripheral nerve injury	Radiculopathy	Postherpet .neuralgia	Trigeminal neuralgia	Total
Demographics						
number of patients	164 (49%)	79 (24%)	61 (18%)	23 (7%)	9 (3%)	336 100%)
female	88 (54%)	45 (57%)	24 (39%)	7 (30%)	1 (11%)	165 (49%)
age (years: mean, ± SD)	62 ± 15	47 ± 11	59 ± 12	69 ± 12	54 ± 18	58 ± 15
Subgroups (based on QST-	**results)**					
normal sensation	32 (20%)	20 (25%)	18 (30%)	7 (30%)	2 (22%)	79 (24%)
loss of thermal sensation	24 (15%)	14 (18%)	13 (21%)	4 (17%)	0 (0%)	55 (16%)
loss of mechanical sensation	38 (23%)	12 (15%)	14 (23%)	2 (9%)	3 (33%)	69 (21%)
loss of mech. and thermal sens.	70 (43%)	33 (42%)	16 (26%)	10 (43%)	4 (44%)	133 (40%)

Table 1. Characteristics of patients and QST results.

Values are given as n (%). QST, quantitative sensory testing.

Figure. 1

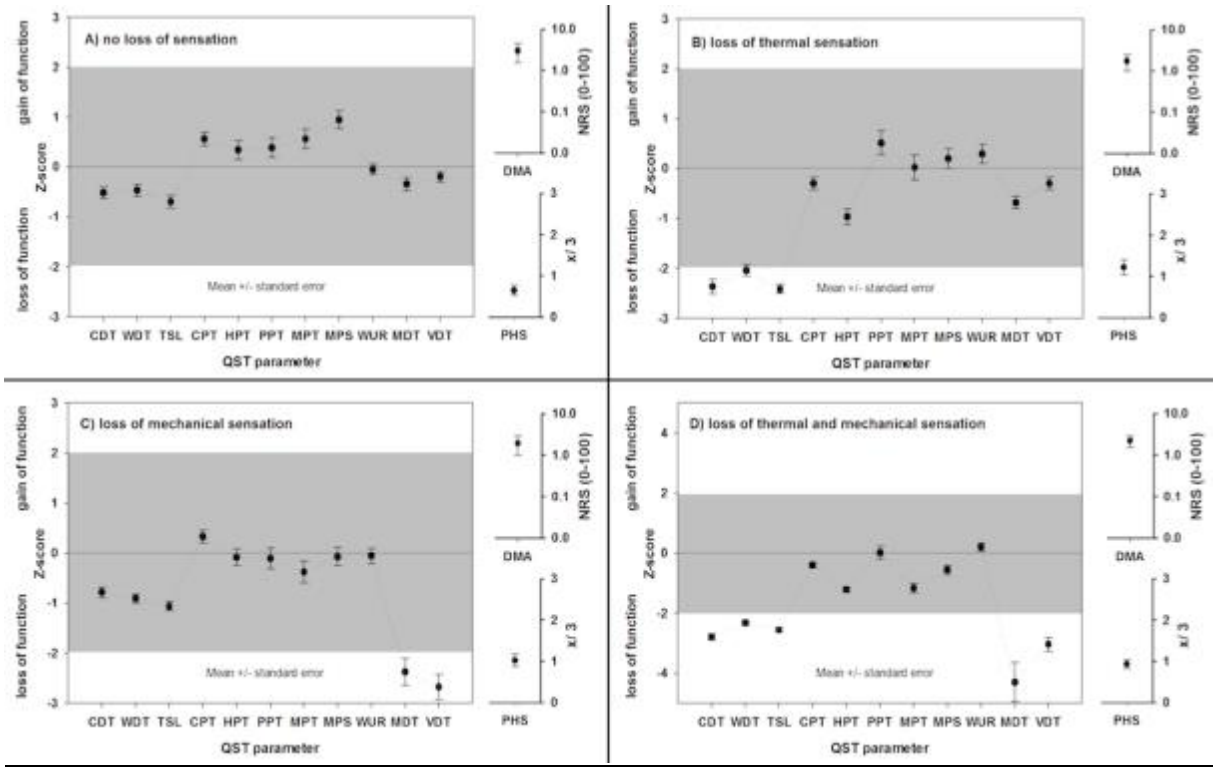

Figure 1. QST–z-profile separately for patients with no loss of sensation (A), loss of thermal sensation (B), loss of mechanical sensation (C), and loss of thermal and mechanical sensation (D). Values are presented as mean 6 SEM. Values between 21.96 and 11.96 represent the 95% confidence interval in an age-matched and sex-matched healthy control population, tested at the same body region. Values below 0 indicate a loss of function (eg, increased detection thresholds), whereas values above 0 indicate gain of function (eg, decreased pain thresholds). CDT, cold detection threshold; CPT, cold pain threshold; DMA, dynamic mechanical allodynia; HPT, heat pain threshold; MDT, mechanical detection threshold; MPS, mechanical pain sensitivity; MPT, mechanical pain threshold; NRS, Numerical Rating Scale; PHS, paradoxical heat sensations; PPT, pressure pain threshold; QST, quantitative sensory testing; TSL, thermal sensory limen; VDT, vibration detection threshold; WDT, warm detection threshold; WUR, wind-up rati

Table 2

Pain intensity					
	Normal sensation	Loss of thermal sensation	Loss of mechanical sensation	Loss of therm. + mech. sensation	p*
Current	4.8 ± 2.2	5.2 ± 2.3	4.6 ± 2.0	5.3 ± 2.2	0.126
maximum (4 weeks)	7.7 ± 1.8	7.9 ± 1.8	7.7 ± 1.6	7.8 ± 2.0	0.943
mean (4 weeks)	5.8 ± 1.9	6.1 ± 1.8	5.6 ± 1.8	6.2 ± 2.0	0.131
Current medication					
NSAID	7 (9%)	13 (24%)	11 (16%)	30 (23%)	0.058
SNRI	9 (12%)	6 (11%)	10 (14%)	13 (10%)	0.798
SSRI	3 (4%)	5 (9%)	7 (10%)	21 (16%)	0.055
Anticonvulsants	26 (33%)	19 (35%)	16 (23%)	45 (34%)	0.408
Tricyclic	18 (23%)	15 (27%)	22 (32%)	52 (39%)	0.089
opioid	13 (17%)	13 (24%)	13 (19%)	41 (31%)	0.081
other	5 (6%)	4 (7%)	9 (13%)	17 (13%)	0.359

Table 2. Pain intensity and medication in relation to sensory loss.

Mean, maximum and current pain intensity are presented as mean ± standard deviation (0 - 10 numerical rating scale). For medication, multiple answers per patient were possible. *Result of a two-way ANOVA (pain) or chi-squared test (medication).

Figure 2.

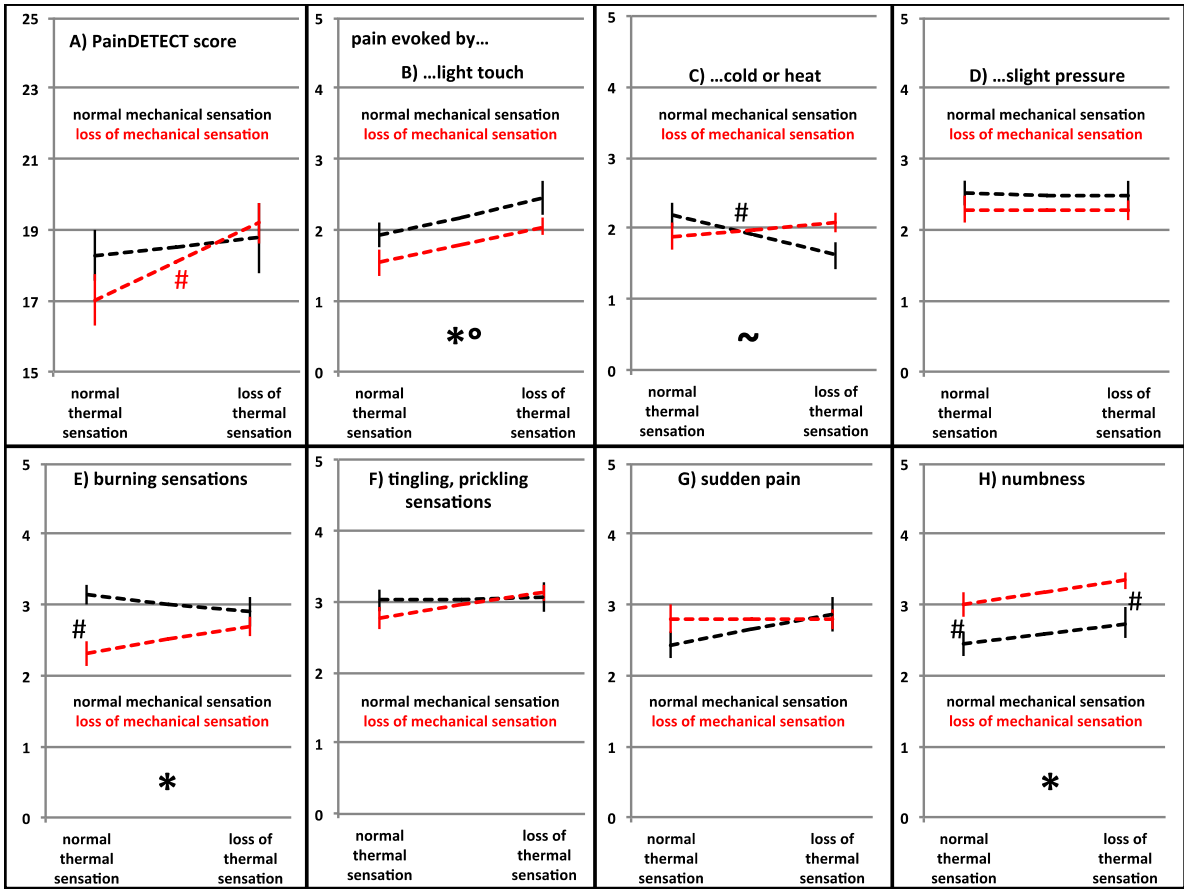

Figure 2. Interaction plot for effects of small and large fiber deficits on total PDQ score (A) and single PDQ item scores of evoked pain (B–D) and spontaneous sensations (E–H), presented as mean 6 SEM. For each item, the values on the left end of each line indicate normal small fiber function (no loss thermal sensation), and values on the right indicate impaired small fiber function (loss of thermal sensation). Black lines indicate normal large fiber function (no loss of mechanical sensation), and red lines indicate impaired large fiber function (loss of mechanical sensation). *Significant large fiber effect, °significant small fiber effect, ;significant interaction effect in the analysis of variance (P , 0.05; Table 3). #Significant post hoc test between patient groups with normal vs loss of mechanical sensation (#on the left or right end of the graph) or between patient groups with normal vs loss of thermal sensation (#in the middle of the graph). PDQ, painDETECT Questionnaire.

Effects of loss of sensation QST phenotype on PDQ items

The values for the overall PDQ score (Fig. 2A) are very similar among the four subgroups. Consequently, there are no significant effects in the ANOVA (table 3).

Table 3.

	model	small fiber effect	large fiber effect	small*large interaction
PDQ Score	1.69 (0.168)	3.08 (0.080)	0.29 (0.593)	1.24 (0.267)
Burning sensation	**4.24 (0.006)**	0.19 (0.665)	**8.85 (0.003)**	3.11 (0.079)
Prickling sensation	0.90 (0.440)	1.60 (0.207)	0.29 (0.590)	1.00 (0.318)
Sudden pain	0.73 (0.536)	1.18 (0.278)	0.63 (0.428)	1.18 (0.277)
Numbness	**6.98 (0.000)**	3.64 (0.057)	**11.59 (0.001)**	0.02 (0.898)
Light touch	**3.16 (0.025)**	**7.82 (0.005)**	**4.43 (0.036)**	0.00 (0.953)
Cold or heat	1.62 (0.184)	1.04 (0.308)	0.16 (0.687)	**4.25 (0.040)**
Slight pressure	0.64 (0.589)	0.03 (0.858)	1.39 (0.239)	0.01 (0.941)

Values significant on P , 0.05 are given in bold. PDQ, painDETECT Questionnaire.

Table 3. Results from the 2-way analysis of variance analyzing the effects of impaired small or large fiber function (abnormal loss of thermal or mechanical sensation) for the PDQ score and each PDQ item presented as F value (P value).

4.Discussion

Although loss of nerve fiber function is an important sign of neuropathy, sensory loss is not represented well in the existing screening tools, possibly because they were created before the new definition of NP emphasized the importance of the clinical sensory examination (Treede et al. 2008). The present analysis reveals that the overall PDQ score, the primary outcome measure, is not related to presence or absence of sensory loss as determined by QST. However, four individual PDQ items were partly sensitive enough to reflect loss of small or large fiber function: numbness, burning sensations, pain evoked by light touch, pain evoked by cold or heat. Whereas numbness and burning sensation reflect perceptions without external stimulation, the other two items may be considered to reflect self- examination by the patient (Bennett et al. 2007).

Subjective report of numbness

In our data, "numbness" is more often reported by patients with loss of mechanical sensation than by those with intact mechanical perception. This is consistent with the interpretation that the subjective feeling of numbness is related to loss of large-fiber or dorsal column functions, for example in a case of unilateral damage to the spinal cord (Geber et al. 2009), where numbness was reported for the side with tactile loss and not the contralateral side with thermal loss. While our data support this concept, the association was not strong enough to allow identification of patients with large fiber loss using this PDQ item.

Subjective report of burning

Reports of "burning sensations" were most frequent in patients with intact mechanical and thermal sensitivity according to QST. This is also the only group where heat pain testing revealed heat hyperalgesia in QST (Fig. 1a), whereas thermal sensory loss was associated with hypoalgesia to heat (Fig. 1b and d). These observations are consistent with the concept that discharges from "irritable nociceptors" may lead to burning sensations (Freynhagen, Baron, Tölle, et al. 2006, Demant et al. 2014). In previous studies, burning pain (and burning sensation) has been discussed as being associated with loss of thermal sensation (Lauria et al. 2012). Unexpectedly, there was no difference between patients without sensory loss and patients with isolated loss of thermal sensation on the item burning sensations. Based on the thermal grill illusion and on observations in patients with multiple sclerosis it had been suggested that burning pain may be due to a disinhibition of a heat-sensitive neural pathway that is normally suppressed by a cold-sensitive pathway (Craig and Bushnell 1994, Hansen et al. 1996). Our observations are at variance with this concept of a disinhibition at the thalamo- cortical level.

Self-report of pain evoked by light touch

The mean report of "pain evoked by light touch" is decreased in patients with mechanical sensory loss, and increased in patients with loss of thermal sensation in QST. Pain evoked by light touch is associated with static or dynamic mechanical allodynia, which has been described in patients with loss of thermal sensation (Heij et al. 2012, Hoeijmakers et al. 2012, Langley et al. 2012).

Sensory phenotype, medication and pain intensity

Pain intensities did not differ significantly among the groups of sensory phenotypes. There are two reasons likely underlying this finding: 1) QST is not able to assess pain intensity itself, and loss of sensation is not correlated with pain intensity. 2) QST and parts of the PDQ describe evoked types of pain, while NRS and the rest of the PDQ capture ongoing pain. Medication also did not differ among the sensory phenotype groups, though there was a tendency for the use of NSAIDs and opioids to be associated with loss of thermal sensation and the use of tricyclic antidepressants and SSRIs to be associated with the large loss of mechanical sensation (all $p < 0.1$). These patterns may influence the results obtained, as pharmacological treatment has the potential to influence pain qualities and sensory descriptors (Mainka et al. 2016). The broad spectrum of analgesics taken reflects the situation in which the patients were recruited: when referred from primary care, where their pain could not be managed.

5. Outlook and Conclusions

NP results from heterogeneous etiological, genetic and environmental causes, possibly leading to different profiles in sensory testing and questionnaire data (Baron et al. 2010, 2012). This, in turn, leads to difficulties in predicting the outcome of currently available therapies (Baron et al. 2010). Several recently conducted proof-of-concept-controlled trials for promising drugs in clinical development have failed to demonstrate an effect superior to placebo (Katz et al. 2008, Dworkin et al. 2012). The reason for this may be that these drugs are aimed at specific targets that may only be relevant in a subgroup of patients with NP, leading to the inclusion of a large group of patients who are unlikely to respond to a drug targeting a pathomechanism that is not underlying their pain condition (Cruccu and Truini 2009, Demant et al. 2014, Mainka et al. 2016). Since pathophysiological mechanisms cannot readily be identified in patients, surrogate measures have been suggested that are likely to reflect underlying pathophysiological mechanisms: e.g., a classification strategy based on the functional gain or loss of mechanical and thermal sensations (LoGa) assessed by QST was introduced (Maier et al. 2010). Furthermore, PainDETECT has been used for sub-grouping of patients according to their pattern of sensory abnormalities. These studies used a hierarchical cluster analysis for segmentation and identified five subgroups of patients with diabetic painful neuropathy, postherpetic neuralgia and painful radiculopathy according to their sensory symptoms (Baron et al. 2009, 2012). Therefore, PainDetect may provide an easy and beneficial way of distinguishing patients according to their sensory profile. Patients could benefit from an early identification of NP and sensory dysfunction phenotype and could avoid insufficient treatment. This approach also could help in deciding whether further investigations are necessary, which may also reduce costs for the health care system.

Questionnaires like the painDETECT are easy-to-use tools in daily clinical practice for gathering information on subjective reports and self-examination (Bennett et al. 2007), whereas bedside sensory testing and QST are validated methods to evaluate the sensory profile in selected pain areas in patients with NP. In contrast to, e.g., conventional nerve conduction studies, both bedside sensory testing and QST can assess the function of thin myelinated and unmyelinated (small) nerve fibers. QST and bedside sensory testing address the diagnostic dilemma, i.e. that patients suffering from isolated loss of small fiber

function cannot be identified by a standard neurological examination (Heij et al. 2012) or nerve conduction studies, which cannot distinguish between healthy subjects and patients suffering from neuropathies with isolated loss of small fiber function (Mendell and Sahenk 2003, Hoitsma et al. 2011). Additionally, QST is suitable for assessing pain thresholds, detection thresholds to non-painful stimuli, and evoked pain, but is unable to capture spontaneous pain, as are conventional electroneurographic techniques. Other diagnostic methods like skin biopsy or corneal confocal microscopy (CCM) reveal information about the structure of small nerve fibers only (Heij et al. 2012, Hoeijmakers et al. 2012, Lauria et al. 2012).

The development of specialized questionnaires for the detection of neuropathic pain phenotypes may improve clinical practice in the treatment of NP outside specialized centers. A simple tool would be beneficial in assisting the primary care physician to select patients needing referral to a specialist for further testing. At present, such screening tools are not sensitive enough to document sensory loss, which is an important criterion in diagnosing neuropathic pain (Treede et al. 2008). It is important to note that QST or skin biopsy cannot be replaced but only supported by such screening tools, or rather a suggestion can be made, if a skin biopsy or QST would be useful. An additional improvement could be a validated and reliable battery of bedside testing(Spiegel et al. 2003) which could be compared in terms of sensitivity and specificity to both pain questionnaires and QST. Such an approach would go far towards working out the value of all three methods in relation to each other.

In conclusion, these results demonstrate that the PDQ score is not sensitive enough to distinguish different types of sensory loss in patients with NP. While our results indicate that there are differences in the responses to four items between groups of patients with one or the other kind of QST-detected loss of sensation, they do not provide clear information on an individual patient basis, as the mean differences are comparably small (about one point on a zero to five scale) and show a huge overlap. PDQ is unable to clearly separate between patients with varying types of loss of sensation, because its questions for loss of function are limited to numbness (and are completely missing in other pain questionnaires), while loss of function can be detected for all sensory qualities in QST. A more complex analysis, identifying items that differentiate between types of sensory loss in a new battery of questions would be an interesting approach for a future study. Some domains may give more detailed information if they are divided into two items, e.g., the item cold or heat pain, one for pain evoked by cold and one for pain evoked by heat. Separate items for the different forms of hyperalgesia or allodynia might also be of value. Additionally, questions about autonomic disorders, which commonly appear in patients with small fiber neuropathy (Lacomis 2002), would be helpful. Pursuing this next step in the development of a convenient screening tool could help identify different phenotypes involved in NP.

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Chapter 3.

Complex Regional Pain Syndrome in Children.

I. **A Multidisciplinary Approach and Invasive Techniques for the Management of Nonresponders**

II. **IInvasive Management for Paediatric Complex Regional Pain Syndrome: literature review and personal experience.**

I. A Multidisciplinary Approach and Invasive Techniques for the Management of Nonresponders

1. Introduction

Complex regional pain syndrome (CRPS) is a term refined by the International Association for the Study of Pain (IASP) to describe disorders characterized by spontaneous or stimulus-induced pain that is disproportionate to the inciting event (Swart et al. 2009, Bruehl 2010, Borchers and Gershwin 2014) . The disease often includes a wide variety of autonomic and motor disturbances in highly variable combinations (Merskey 1994a, 1994b) in addition to a mixture of noxious sensations (positive symptoms) and sensory loss (negative symptoms) (Swart et al. 2009, Gierthmühlen et al. 2011, Marinus et al. 2011).

CRPS has been extensively studied in adults, but studies in children are scarce. Previously, it was doubtful that this condition even existed in children; however, numerous recent articles have reported CRPS in children (Finniss et al. 2006, Logan et al. 2013). Furthermore, several authors have highlighted differences in the pediatric presentation compared to that of adult CRPS (Tan et al. 2009). Approximately 90% of reported cases are girls between 8 and 16 years of age, with the lower limbs most commonly affected. Leading symptoms are intensely burning pain, along with cold and mechanical allodynia, dysesthesia and paresthesia. Additionally, signs of autonomic dysfunction, movement problems and psychological difficulties are also regularly present (Sherry and Weisman 1988, Berde and Lebel 2005, Finniss et al. 2006, Stanton-Hicks 2010, Logan et al. 2013).

Not only does diagnosing CRPS pose a significant challenge, but the timing of the diagnosis can also determine the prognosis (Murray et al. 2000, Lee et al. 2002, Berde and Lebel 2005, Finniss et al. 2006). Furthermore, prompt and accurate management is vital, since the cornerstone of therapy is to restore function of the affected limb. Recognized therapies include a combination of pharmacotherapy, physical therapies and psychotherapy where appropriate (Lee et al. 2002, Wilder 2006, Zernikow et al. 2012, Chopra and Cooper 2013, Borchers and Gershwin 2014). There is evidence that the pediatric population responds better to non-invasive approaches (Maillard et al. 2004) . As a result, this style of management is growing across Europe and the United States (Wilder 2006, Logan et al. 2013) . Nonetheless, an exact treatment model or algorithm has not yet been established. Unfortunately, not all patients respond successfully to conservative management, making further interventions a necessity. Many patients who fail to progress with physical therapy may require additional or more aggressive pain therapy, such as spinal cord stimulation (SCS) or intra-spinal analgesic infusion (Hord and Oaklander 2003, Stanton-Hicks 2010, Taylor et al. 2012) . The significance of invasive therapies in children who do not respond to conventional treatments or medications has not been established, although most therapies used in adult CRPS have been tested in children, including spinal stimulation or drug infusion, TENS and sympathetic blockade under general anesthesia. One can find numerous reports in the literature demonstrating success using these procedures (Kemler, de Vet, et al. 2008, Kemler et al. 2010, Kato et al. 2011, Olsson et al. 2012, Martin et al. 2013) , providing doctors with further alternatives when the non-invasive options are not

enough. Consequently, we hypothesized that children who do not respond adequately to conservative measures may have the same opportunity to reduce their symptoms with invasive treatments.

The present article reports on the course and management of 10 children diagnosed with CRPS who did not respond successfully to conservative pain management therapies presenting to our Pain Clinic.

2. Methods

This is a clinical series reporting on 10 cases of children (5 males and 5 females) between 8 and 13 years old who presented to *the Pain Treatment Unit of the Hospital Regional Universitario de Malaga* between July 2010 and May 2014. These patients had been diagnosed with CRPS, but previous pain therapy was not successful. Approval from the hospital's institutional Review Board was obtained before the study was conducted. For this study all CRPS patients were recruited and diagnosed at the pain unit. Next we followed a treatment pathway using conventional management first and invasive treatments if the patients did not respond, described in detail below. Follow up appointments were carried every fourteen to twenty days until resolution of the symptoms or after a year from the diagnosis at the pain unit.

All patients between 5 and 16 years old who were evaluated for long-lasting limb pain and possible CRPS in our Pain Treatment Unit from the May 2010 to May 2014 were included in a process to determine if they met the modified IASP Budapest criteria for the diagnosis of CRPS (Harden et al. 2007) . The final diagnosis was made in all cases by a senior pain medicine consultant using the clinical diagnostic criteria for CRPS proposed by the Budapest IASP consensus group; this was necessary for inclusion in the study. Children with neuropathic or limb pain who did not meet these criteria were excluded from the study. The 10 children meeting the Budapest criteria also matched the less rigorous current IASP diagnostic criteria for CRPS.

In this study, all included patients were referred to the Pain Treatment Unit because of limited or no response to non-invasive treatment (physical therapy, NSAIDs and acetaminophen). All patients were referred to the Pain Treatment Unit by the services of Traumatology and Pediatric Rheumatology, with a median time for the referral of 18 weeks. The medical and clinical documentation of the patients who were referred to our pain unit and who accomplished the diagnosis of CRPS were reviewed since the presumptive diagnosis of CRPS commenced at the referring service. These patients were followed for at least 12 months after the diagnosis was made at the pain unit. The length of time taken to make the diagnosis was calculated from the onset of symptoms. Once diagnosis was made the length of time to recovery was calculated. Full recovery was defined as recovery with no recurrence of symptoms within the initial 5 months following resolution. Recurrences after this time were considered new episodes.

Treatment approach.

In this study, the same treatment protocol was followed for every patient after exhaustive clinical investigations *(Figure 1a)*. After the initial consultation and diagnosis, the first option was always pharmacological treatment for neuropathic pain, together with physiotherapeutic management and psychological therapy where needed. The physiotherapy treatment prescribed was exercise to facilitate

motion, strength, and proprioception among other qualities, together with sensory desensitization. Psychological treatment entailed daily individual and group-based cognitive behavioural therapy (CBT). Pharmacological treatments included gabapentin, pregabalin or antidepressants such as amitriptyline, prescribed alone or in combination with other analgesic drugs such as tramadol. Additionally, if the patient presented intense allodynia and/or severe hyperalgesia, the capsaicin 8% patch was offered as an option to support pharmacological treatment. For those children who did not respond to this type of treatment after a period of 3 to 5 weeks, a more invasive treatment was chosen. Treatment responsiveness was determined by a decrease of at least 33% on the Visual Analogue Scale (VAS) and functional improvement determined independently by the patient, the physiotherapist and one of the consultants at the pain unit. At this point, the first step was neuraxial analgesia. Under general anaesthesia, we placed a lumbar epidural catheter for bupivacaine infusion of two weeks *(Figure 1b)*. After this time, the catheter was removed and the subject was evaluated. The VAS scale and motor dysfunction were assessed and compared with previous records, and possible side effects were also recorded. If pain persisted, our next step was to surgically place a spinal cord stimulator under general anaesthesia. Correct positioning of the electrode and stimulation parameters were set once the patient was awake and fully recovered from the anaesthesia. A trial stimulation with a temporary percutaneous extension was performed for about two weeks before permanent implantation of the pulse generator.

Outcome measures included spontaneous and evoked pain (VAS), the presence of dysaesthesia, allodynia, hyperalgesia, sensitivity to cold, dysautonomic signs, motor dysfunction (Functional Disability Inventory (FDI)) (Kashikar-Zuck et al. 2011) , ability to weight-bear, analgesic consumption, and school attendance. All of these items were assessed at periodic appointments every 2 weeks except for motor dysfunction, measured at the first visit, at month 6 and at the end of the follow-up, i.e. month 12. FDI classifies the physical disability as minimal, moderate or severe. The appointments were made between the diagnosis and the resolution of symptoms in order to follow the evolution closely, modify the treatment if needed and to detect any possible side effects.

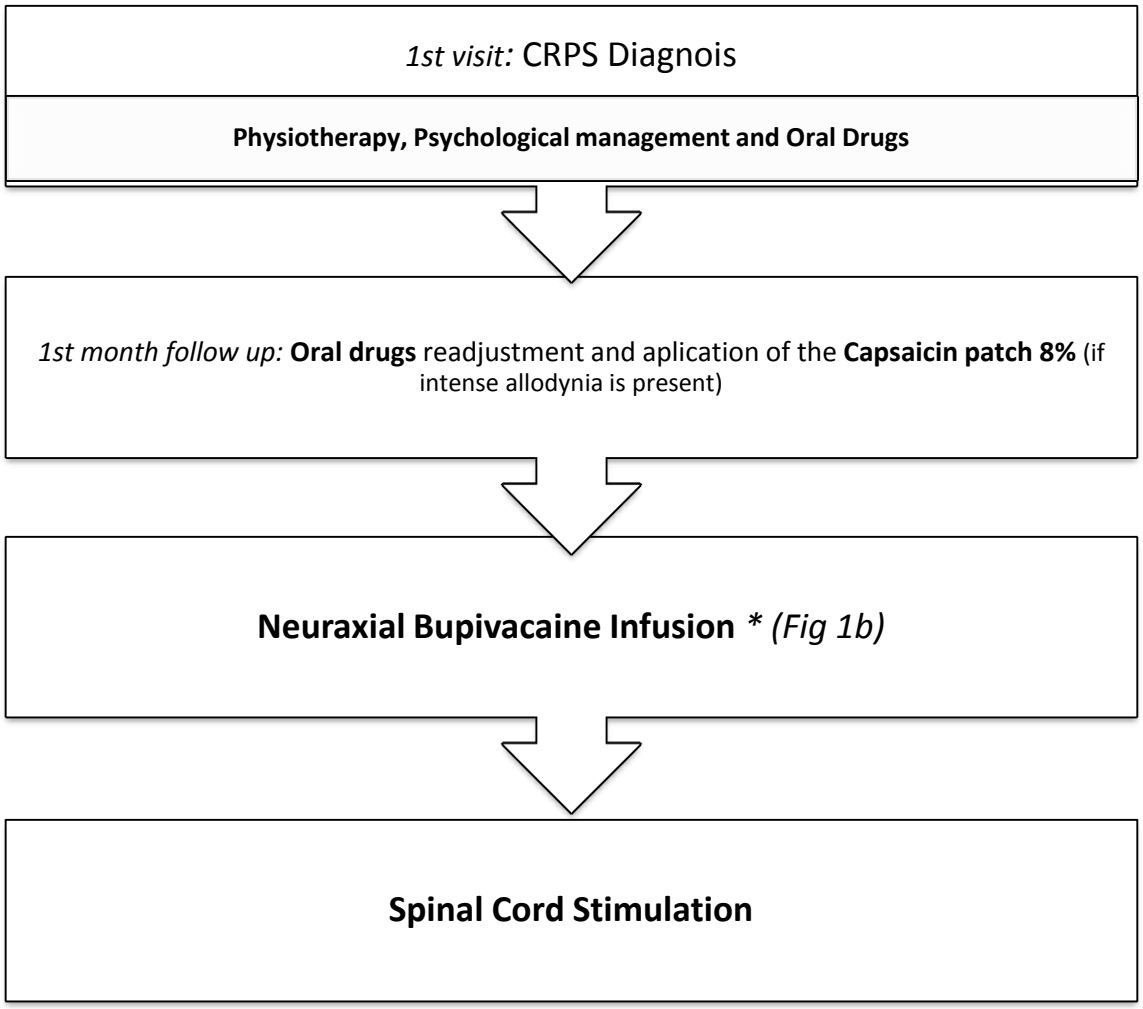

Figure 1: Treatment approach. At the first visit CRPS diagnosed is made and a new treatment plan is recommended. This gathers oral medication as gabapentinoids, antidepressants and analgesics. Weekly follows up allow us to readjust the medication and add topical capsaicin if allodynia is intense. 40 to 50 days after conservative treatment has been started, if this has not been effective Neuraxial bupivacaine is tested over 2 weeks (Figure 1b). If neuraxial analgesics are not effective either, SCS is tested and finally implanted if the treatment is successful.

Figure 1b: Epidural local analgesic infusion. All patients who were going to receive this treatment were hospitalized the day before. The epidural catheter was inserted via the L3–L4 inter-space and advanced into the epidural space using an 18G Thuoy needle under fluoroscopy guidance. This technique was performed under general anesthesia and with the patient in *lateral decubitus* position. The catheter was then tunneled subcutaneously and connected to the pump. Pump-catheter system integrity was verified post-operatively. Treatment was started after the patient got recovered from the anesthesia, Bupivacaine 0.25% at 2ml/hour. This dose was titrated until reach the 4ml/hour over 72hours. All patients continued at the hospital for 24 hours after the intervention prior discharge. This treatment continued for 14 days. After this time the epidural catheter got carefully removed.

Case Reports:

Case 2

Female, 13 years old. Without any trauma or other reasonable cause, she started to have pain in both feet 16 weeks before she was referred to the pain unit. CRPS was diagnosed and we rapidly started extensive psychiatric treatment and physiotherapy *(VAS 8/10)*. At that time, she had developed contractures and was confined to a wheelchair *(FDI 41)*. Pharmacological treatment was started as well, using the capsaicin 8% patch to minimize allodynia. A few weeks later, she reported progressive relief of her pain and motor disturbances. At follow-up 12 weeks later, she had no pain or dysautonomy and was contracture free *(VAS 0/10, FDI 5).* Neither mechanical nor dynamic allodynia were evoked after 2 weeks of the initial treatment with the capsaicin patch; this was maintained until CRPS resolution.

Case 5

Female, 8 years old. The patient was diagnosed with CRPS after suffering a minor trauma to her left foot while playing at school. CRPS was resolved successfully with pharmacological treatment in a few days. However, 4 weeks later, without any new trauma or other reasonable cause, she started to have pain on the contralateral limb. Oedema, redness, dysaesthesia, paraesthesia, allodynia and motor problems were also present at the time of the diagnosis. First line therapies of the management plan were implemented; however, the reduction of symptoms was modest, and the VAS score decreased by only by two points while the FDI score remained over 40 (severe). Therefore, we implanted an epidural catheter under general anaesthesia for continuous epidural infusion of bupivacaine *(Figure 1b)*. After 2 weeks, the pain, dysautonomic and motor symptoms were significantly reduced. At this time, the infusion was stopped and the catheter was removed. She reported a reduction in spontaneous pain from *VAS 9/10 to 1/10*. She continued with oral medication and further physiotherapy, leading to the complete cessation of pain for 12 more months. She is still pain-free in both feet 18 months later. Motor dysfunction is minimal and both autonomic and sensory abnormalities disappeared altogether in both feet.

Case 7

Male, 9 years old. After a minor ankle sprain while playing soccer, the patient developed persistent pain *(VAS 7/10)*, severe allodynia and dysautonomic signs in the affected foot and lower leg. With our first line management therapies, including the 8% capsaicin patch, he reported a significant reduction in allodynia but insufficient relief of pain *(VAS 5-6/10)* and motor disability *(FDI severe dysfunction)*. Neuraxial analgesia with a 2-week infusion of bupivacaine successfully diminished his pain and allowed him to recover full mobility of the limb once again *(FDI 10)*. Reductions were achieved in the VAS score and functional disability, i.e. from 7 and 9 for evoked pain down to 0 and from severe to minimal, respectively, with this treatment protocol. After the epidural catheter was removed and the infusion was stopped, the subject continued to be pain-free until the last follow up, 24 months later *(VAS 0/10, FDI 3*).

Case 9

Female, 13 years old. CRPS in the lower right limb developed a few days after an ankle fracture. The patient did not respond adequately to physical and pharmacological treatment during the first 30 weeks. She presented a *pain VAS score of 8/10* and severe motor impairment. She was thus referred to our pain unit where an epidural bupivacaine infusion was used to treat her pain. The pump was stopped and the catheter was removed 2 weeks after, obtaining excellent results. The girl reported pain relief with a *VAS for evoked pain from 9/10* down to *2/10*. However, the symptoms reappeared after the infusion was stopped. Following this, an SCS trial device was implanted with excellent results for 2 weeks *(VAS 1/10),* allowing the patient to resume school and physical therapy; the impact of this treatment allowed for the patient to minimize her motor impairment. Two weeks after that improvement, we disconnected the stimulator device and continued with the physical and psychological therapies. Four weeks later, we achieved complete remission of the pain and additional symptoms, so we decided to remove the percutaneous electrode. Twelve months later, the patient remains pain-free and with minimal functional disabilities.

Case 10

Female, 8 years old. She suffered a minor trauma to her left foot 17 weeks before she was referred to the pain unit with severe pain *(VAS 10/10)*, allodynia, dysaesthesia and dysautonomic symptoms. Before her referral, she was treated with transdermic opioids and gabapentin among other analgesics, with poor results. At the pain unit, pharmacological treatment including topical capsaicin patches relieved her allodynia significantly but only modestly reduced her pain by two points on the VAS scale. Continuous epidural infusion of bupivacaine caused a temporary suppression of her spontaneous pain, but it came back when the infusion was stopped. SCS was decided on and about 7 months after the start of the pain, an octopolar paddle electrode was implanted. Three weeks after the stimulation started, the symptoms were minimal and the patient regained complete limb functionality, returned to school and also started taking part in extra-curricular activities *(VAS 0/10, FDI 6)*.

3. Results

Following the management pathway described above, the outcomes were *noteworthy*. This clinical series shows that 9 out of 10 patients had suffered a minor trauma or ankle sprain months before they were referred to the clinic, and presented with high intensity, neuropathic-type pain, along with significant motor and autonomic disturbances since the onset of injury (*Table 1)*. Strikingly, one patient suffered trauma to the limb contralateral to where the symptoms were reported. The treated children had significantly reduced pain and improvement in other symptoms such as allodynia, dysaesthesia, hyperalgesia, sensitivity to cold and dysautonomic signs. CRPS even disappeared in the majority of cases. Although the children were all instructed to assess their pain using a VAS, it was not feasible to achieve this on a regular basis. The patients in our study primarily remained at home and only came to the pain unit for appointments. The children failed to do regular evaluations at home, so data were usually collected only at appointment times. Pain scores (VAS) decreased substantially during the study. The mean VAS for ongoing pain at the first visit was 7.7 (SD 0.9) and for evoked pain was 9.1 (SD 0.93), while 12 months after the first appointment, the mean VAS for the whole group was 0.3 (SD 0.4).

Physical functional disability was also reduced. Our patients showed severe disability at their first visit *(mean FDI: 32.4, SD 7.3*); however, at the end of the study, they presented minimal disability *(mean FDI: 4.8, SD 3.1)*. These data parallel school absences, which declined from 60% to 0% throughout the study.

Within this series of patients, CRPS disappeared with oral medication and physical management in one patient. Three other children alleviated their symptoms by adding the capsaicin 8% patch to their pharmacological and physical management. Capsaicin treatment diminished mechanical and dynamic allodynia in all nine patients in whom it was used. Allodynia that was originally stated as “extremely unpleasant” at the beginning of the treatment in seven out of ten children either disappeared completely or was just mildly unpleasant at the last appointment for all patients.

A second group of children needed further efforts to treat CRPS. Non-invasive management was unsuccessful in alleviating pain in these patients after a few weeks of treatment. Three of these children reduced their pain with two weeks of epidural bupivacaine by continuous infusion. None of these patients experienced a recurrence of symptoms after the epidural catheter was removed.

Finally, three patients who did not respond to systemic medication or to neuraxial analgesia needed the surgical placement of an octopolar paddle spinal cord stimulator to relieve their pain. This last group of patients improved their clinical situation significantly with this treatment, making better physiotherapeutic management possible and reducing and/or abolishing the need for any oral medication *(Table 2).*

Table 1.

Case	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Age (years)	13	13	10	11	8	10	9	11	13	8
Gender	Male	Female	Male	Male	Female	Female	Male	Female	Female	Female
Area affected	RLL	Bilateral	RLL	LLL	RLL	RLL	LLL	LLL	RLL	LLL
Trauma	Mild	No	Mild	Mild	Minor*	Mild	Minor	Mild	Mild	Minor
Time diagnosis-referral (weeks)	28	16	19	17	4	150	95	36	30	17
Ongoing Pain (VAS)	6	8	7	8	8	7	7	9	8	9
Evoked Pain (VAS)	9	10	8	9	10	7	9	10	9	10
Allodynia	+	++	++	+++	+++	++	+	+++	++	++
Motor dysfunction (FDI)	Minimal-Moderate	Severe	Minimal	Moderate	Severe	Moderate-Severe	Severe	Moderate-Severe	Severe	Severe
Dysautonomic symptoms	+	+	+	++	++	++	+	++	+++	+
Other sensory disturbances	+	+	+	+++	++	++	++	+	+++	+

Table 1. Demographics and clinical data of ten cases of children with CRPS

None of our patients reported mild or important secondary effects due to any of the therapies used during the study. Some minor side effects such as transient dizziness, dry mouth or minor pain after surgery were reported in 30% of cases.

Table 2.

Case	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Time diagnosis- referral (weeks)	28	16	19	17	4	150	95	36	30	17
Physical Therapy	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Psychological Therapy	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Pharacological Th.	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Capsaicin 8% Patch	-	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Neuroaxial Bupivacaine infusion	-	-	-	-	Yes	Yes	Yes	Yes	Yes	Yes
SCS	-	-	-	-	-	-	-	Yes	Yes	Yes
Follow up 12 months after the first visit at the Pain Unit.										
Time referral – "CRPS free" (weeks)	22	12	19	8	30	32	24	48	21	39
Ongoing Pain (VAS)	0	0	1	0	0	0	0	1	0	0
Allodynia	No	No	No	No	No	No	No	No	No	No
Motor dysfunction (FDI)	Minor	Minor	Minor	Minor	Minor	Minor	Minor	Minor	Minor	Minor
Taking Drugs	No	Yes	No	Yes	Yes	No	No	Yes	No	Yes
School absence	No	No	No	No	No	No	No	No	No	No

Table 2. - Therapies prescribed and clinical evaluations after a 12 moths follow up.

4. Discussion

Complex regional pain syndrome is usually represented by a complex clinical presentation and (Merskey 1994a, 1994b, Meier et al. 2006, Marinus et al. 2011, Gierthmühlen et al. 2014) a pathophysiology that seems to be multifactorial in nature, characterized by an aberrant host response to tissue damage. Most of the clinical features of this condition are apparent in the confluence of three major pathophysiological pathways: vasomotor dysfunction, aberrant inflammatory mechanisms and maladaptive neuroplasticity. The clinical heterogeneity of the disorder is indicative of the inter-individual variability in the activation of these pathways after tissue injury (Jänig 2009, Coderre and Bennett 2010, Marinus et al. 2011) .

Several authors have highlighted differences in the picture presentation compared to that of adults with CRPS (Tan et al. 2008) *(Table 3).* Abnormal intensity and burning pain, in addition to mechanical allodynia, dysaesthesia and paraesthesia are present in nearly 100% of the cases reported in the literature. Signs of autonomous dysfunction such as oedema and discoloration are present along with a significant reduction in movement range of the affected limb (Berde and Lebel 2005, Finniss et al. 2006, Meier et al. 2006, Fitze 2011, Logan et al. 2013) . The symptoms displayed in our patients were in most cases the majority of those described above. The complexity of CRPS shown in this group of children was outstanding as the majority took a long time to be diagnosed and treated appropriately for their condition.

In our experience, the prognosis of the disorder substantially depends on an early diagnosis. Most studies agree with this finding (Murray et al. 2000, Lee et al. 2002, Berde and Lebel 2005, Finniss et al. 2006). Low et al. showed that children who get a prompt diagnosis (less than 12 weeks) and therefore rapid treatment achieved a quicker and more successful remission of CRPS when compared to those whose diagnosis was delayed (10.6 and 21.5 weeks) (Low, Ward, and Wines 2007a) . In spite of this, Murray et al. could not corroborate the relationship between early onset of treatment and early recovery (Murray et al. 2000) . Still, we support that early detection and diagnosis, using standards such as the Budapest criteria to ensure this, can attenuate the possibility of permanent suffering, since delayed diagnosis may result in lifelong pain, functional deficiency and psychological complications (Harden et al. 2007, Fitze 2011) . Unfortunately, children are often diagnosed in a delayed fashion because CRPS is still a rare and unknown condition in this population. Frequently, children will have seen multiple health care providers before a formal diagnosis is made, eventually leading to the symptoms described above (Kachko et al. 2008).

Several characteristics have been identified that differentiate CRPS in children and adults *(Table 3)* (Tan et al. 2009, Logan et al. 2013) ; however, these are not only clinical. During childhood, CRPS usually appears in the lower extremities, whereas the upper limb is most commonly affected in adults (Berde and Lebel 2005, Harden et al. 2007) . To further this point, Tan et al. demonstrated in a retrospective study of young patients that the upper limb was affected in only 23.3% of these patients versus the 72.6% who had an affected lower limb (Tan et al. 2008). In our study, the difference was even more notable as all patients presented CRPS in the lower extremities. Another difference identified in the literature between adults and children is that females are more often affected than males (7:1) (Harden et al. 2007, Low, Ward, and Wines 2007a) . However, we did not find this ratio, as our study had an equal proportion of male to female patients. Finally, another interesting detail found in this study is that children show complete recovery of

any lost functionality after correct management, whereas adults retain some disability after the symptoms are resolved (Stanton-Hicks et al. 1998, Sherry et al. 1999, Berde and Lebel 2005, Bruehl 2010) .

Table 3.

Characteristic	Adult	Paediatric
Age*	45	12
Gender ratio	Male predominance	Female predominance
Extremity affected	Upper	Lower
Trauma	Mild- Severe	Minor- Mild
Limb temperature	30% cooler	70% cooler
Oedema	40%	75%
Prognosis	Variable, long term disability	Excellent recovery in most cases
Relapse rate	10%	30%

Table 3. Adult vs. Paediatric CRPS characteristics.

* Mean age at presentation of the symptoms

Most clinicians agree that the best possible prognosis is achieved with an diagnosis and treatment (Murray et al. 2000, Lee et al. 2002, Finniss et al. 2006) . Additionally, the medical community agrees that the cornerstone outcome should be the restoration of function. Acknowledged therapies include a combination of pharmacotherapy, physical therapy and psychotherapy where appropriate (Lee et al. 2002, Wilder 2006, Zernikow et al. 2012, Chopra and Cooper 2013, Borchers and Gershwin 2014) . Several studies have shown that early mobilization of the affected limb assisted with cognitive behavioural techniques is the most important part of the management process in childhood (Sherry et al. 1999, Lee et al. 2002, Wilder 2006) . In our experience, this has remarkable importance, but so does the use of medication as it was shown to be indispensable in pain reduction. This is why we provide multidisciplinary management, including physiotherapy, psychotherapy and pharmacological treatment as soon as patients arrive at the pain clinic.

Among the drugs used to treat CRPS, NSAIDs, acetaminophen, tramadol, tricyclic antidepressants and gabapentinoid anticonvulsants are frequently recommended (Low, Ward, and Wines 2007a, Chopra and Cooper 2013) . However, despite different combinations and dose escalation of these drugs, some patients do not improve in terms of sensations such as allodynia in severe cases. With the aim of reducing this, we began treatment with a capsaicin 8% patch in those who presented severe allodynia. The capsaicin 8% patch is not currently FDA or EMA approved for use in children. We meticulously explained to the children

and their families the mechanism of action of the patch in adults, the risks associated with its use, secondary effects, different therapeutic options and, more importantly, our aims and hypothesis. After this, the parents of seven children signed the consent form approving the use of the patch. The capsaicin patch was applied in the seven children, providing significant relief for allodynia, hyperalgesia and dysaesthesia in five of them. In three patients, the capsaicin 8% patch together with oral medication and physical and psychological treatment eradicated all symptoms. Here, the application of the patch was the final and decisive step in their treatment. Notably, none of the children treated suffered any side effects. This new and improved topical formulation has emerged as an effective tool to treat chronic refractory pain in adults; however, to our knowledge, it has not yet been documented for use in CRPS during childhood. This is an important tool that should be incorporated as part of a complex analgesic regimen for improving pain management plans in the paediatric CRPS population.

Only those patients who did not improve successfully after being treated with the three-pillar pain management plan described above were candidates for invasive pain therapies. The implications of invasive managements in children who do not respond to conventional treatments are not established and a positive publication bias must be assumed. Nevertheless, there are numerous reports of treatment success using invasive techniques (Kemler et al. 2000, Kemler, de Vet, et al. 2008, Kato et al. 2011, Olsson et al. 2012, Martin et al. 2013) , providing doctors new alternatives when non-invasive options are not enough.

In this study, six patients (60%) required these techniques after exhausting all other possibilities. Pain and motor incapability continued progressing despite non-invasive treatment for more than four weeks at the pain unit. In the operating room and under inhalation anesthesia, we placed a lumbar epidural catheter for bupivacaine infusion, as described in *Figure 1*. Doctors at the Pain Treatment Unit managed the local anaesthetic infusion externally, which was titrated carefully until there was a decrease in pain without side effects. The infusion continued for two weeks, after which the catheter was removed and the subject was evaluated. In three of our patients, pain and other symptoms decreased permanently, co-medication was reduced and physical therapy management helped significantly. On the other hand, in the other three patients, symptoms reappeared after an initial period of improvement.

In the unsuccessful cases, SCS was the next option for treatment. After obtaining consent, we surgically placed an octopolar paddle spinal cord stimulator. This last group of patients improved their clinical situation significantly with this treatment, making better physiotherapeutic management possible and reducing and/or abolishing any oral medication. In the line of this study, we have found several reports on the use of SCS in children (Olsson et al. 2012) . However, no curative effect of SCS has been reported, not even in adults. The mechanism behind the beneficial effects of SCS is basically unknown, but reviews of the present knowledge and some hypotheses relating to CRPS have started to come to light (Linderoth and Meyerson 2010). From our point of view, SCS is a minimally invasive and reversible treatment method that can be useful in the management of otherwise therapy-resistant CRPS presentations in children.

4. Conclusion

This study offers a multidisciplinary management approach to the treatment of CRPS in children for whom the standard treatment was not successful. Because of the severity and rapid progression of symptoms in CRPS, we consider that early diagnosis of the condition together with comprehensive and individualized multidisciplinary treatment offers children with CRPS the best opportunity for complete recovery. Within this management plan, novel drugs should be included, such as the capsaicin 8% patch, in addition to invasive techniques for patients who otherwise do not respond to non-invasive therapies. Thus, a more aggressive approach needs to be attempted. We conclude that further research into CRPS in children is needed and new treatment guidelines are required for those children who do not respond to established management modalities.

II. Invasive Management for Paediatric Complex Regional Pain Syndrome: literature review and personal experience.

1. Introduction

Complex regional pain syndrome (CRPS) is a term defined by the International Association for the Study of Pain (IASP) to describe disorders primarily characterized by spontaneous or stimulus-induced pain that is disproportionate to the inciting event. CRPS has been suggested to be a multifactorial disorder that is related to an aberrant host response to tissue damage (Swart et al. 2009, Marinus et al. 2011) . The disease often includes a wide variety of autonomic and motor disturbances in highly variable combinations (Merskey 1994a, 1994b). The symptoms can be categorized into two groups; positive noxious symptoms, such as hyperalgesia and allodynia and negative symptoms of sensory loss (Swart et al. 2009, Gierthmühlen et al. 2011, Marinus et al. 2011). Usually, patients with CRPS present following moderate or insignificant tissue damage. In the acute phase, the patient can exhibit an extremely painful, red, warm and swollen injured limb. Other potential accompanying features are changes in sweating, hair and nail growth, allodynia and hyperalgesia, and muscle weakness. As the disorder continues, pain spreads, voluntary motor control is reduced in most patients, and negative sensory signs, namely hypoalgesia and hypoesthesia, become more apparent (Bruehl 2010, Maier et al. 2010, Marinus et al. 2011).

CRPS has been extensively studied in adults while studies in children are scarce (Marinus et al. 2011, van Eijs et al. 2011, Gierthmühlen et al. 2014). For a long time it was doubtful that this condition even existed in children, nonetheless within the last few years numerous articles have reported CRPS at young ages *(Table 1)*. However, due to the lack of understanding regarding its precise pathophysiology, reliable diagnostic tests are not available. CRPS diagnosis entirely depends on observable signs and reported symptoms, which have been put together into various diagnostic criteria sets for adults (Stanton et al. 1993, Merskey 1994a, Harden et al. 2007). Unfortunately these diagnostic criteria do not often agree, raising a high degree of uncertainty into CRPS diagnosis. To date specificity and sensitivity of the standard diagnostic criteria sets have not been evaluated for pediatric patients.

As well as posing a significant diagnostic challenge, the timely diagnosis of CRPS can substantially influence the prognosis (Murray et al. 2000, Lee et al. 2002, Berde and Lebel 2005, Finniss et al. 2006). Additionally, prompt and accurate management is key, where the cornerstone is to restore function of the affected limb. Recognized therapies include a combination of pharmacotherapy, physical therapies and psychotherapy where appropriate (Lee et al. 2002, Wilder 2006, Zernikow et al. 2012, Chopra and Cooper 2013, Borchers and Gershwin 2014). Only patients who fail to progress with physical therapy may require additional or more invasive pain therapy, such as spinal cord stimulation (SCS), intra-spinal analgesic infusion or sympathetic blocks (Hord and Oaklander 2003, Stanton-Hicks 2010, Taylor et al. 2012, Logan et al. 2013). Neurostimulation therapy and spinal cord drug infusion have been available since the 1970s and have grown in acceptance in recent years for the treatment of pain disorders of diverse etiology (Knight et al. 2007, Taylor et al. 2012). Today, CRPS in adults is the second-largest indication for the use of SCS in the United States, reaching success rates of up to 70% in pain reduction in CRPS sufferers treated with spinal cord stimulation when properly selected (Stanton-Hicks et al. 1998, Barolat and Sharan 2000). However, the significance of invasive procedures during childhood and adolescence for the

treatment of CRPS patients who do not respond to conventional treatments or medications continues to be unestablished (Zernikow et al. 2015). Several reports in the literature demonstrate success with these procedures, providing physicians (or clinicians) with more alternatives after conventional options fail *(Table 3)*.

The focus of this article is to review the evidence for invasive pain procedures along with presenting a management algorithm for pediatric CRPS, including invasive procedures for patients who do not respond to the conventional first-line treatment.

Intervention	n studies (%) N=31	*n* patients	1980-2000	2000-2015	Reference
Sympathetic blockade (singular or continuous)	15 (48%)	123	7	8	(Doolan and BROWN 1984, Dietz et al. 1990, Wilder et al. 1992, Stanton et al. 1993, Lloyd-Thomas and Lauder 1995, Honjyo et al. 1997, Maneksha et al. 2000, Matsui et al. 2000, Tong and Nelson 2000, Agarwal and Joseph 2006, Meier et al. 2006, Nordmann et al. 2006, Dangel 2008, Kachko et al. 2008, Franklin and Austin 2012)
Spinal drug infusion or epidural catheter	11 (35.5%)	25	0	11 *(100%)*	(Maneksha et al. 2000, Matsui et al. 2000, Tong and Nelson 2000, Ingelmo et al. 2005, Meier et al. 2006, Stanton-Hicks and Kapural 2006, Farid and Heiner 2007, Rodríguez et al. 2007, Kachko et al. 2008, Rand 2009, Kato et al. 2011)
Regional anesthesia	10 (32%)	36	1	8 *(91%)*	(Stanton et al. 1993, Maneksha et al. 2000, Matsui et al. 2000, Suresh et al. 2003, Dadure and Capdevila 2005, Kachko et al. 2008, Carayannopoulos et al. 2009, Kato et al. 2011, Martin et al. 2013)
Intravenous lidocaine	7 (22.4%)	28	4	3	(Buchta 1983, Doolan and BROWN 1984, Honjyo et al. 1997, Di Vadi et al. 2006, Meier et al. 2006, Nordmann et al. 2006, Dangel 2008)
Spinal Cord Stimulation	3 (9.6%)	11	0	3 *(100%)*	(Stanton-Hicks and Kapural 2006, Rodríguez et al. 2007, Olsson et al. 2012)
Surgery	3 (9.6%)	5	3	0 *(0%)*	(Buchta 1983, Ashwal et al. 1988, Parano et al. 1998)
Sympathectomy	2 (6.4%)	28	2	0 *(0%)*	(Buchta 1983, Greipp et al. 1988)
	31(100%)	171			

Table 1. Invasive Interventions for Complex Regional Pain Syndrome

2. Material and Methods

Literature selection

A literature search identified studies relevant to invasive treatments for CRPS in children. Databases used included PubMed, Medline, Web of Science, Embase and Cochrane. Because of the small volume of literature on the pediatric population, database-specific controlled vocabulary (subject headings or index terms) was not used, and keyword searching produced a comprehensive and manageable yield. The following search strategy was used: ((complex regional pain syndrome) OR (CRPS) OR (reflex dystrophy) OR (algodystrophy) OR (causalgia) OR (Sudeck´s atrophy) AND (sympathetic OR neurovascular)) OR ((amplified OR complex OR chronic) AND (neuralgia OR pain) AND musculoskeletal)) AND (therapy OR therapies OR therapeutic)) OR (transcranial AND magnetic AND stimulation) (OR spinal cord stimulation OR neurostimulation OR spinal drug infusion OR intra-spinal therapy OR epidural infusion OR epidural catheters OR sympathetic block OR sympathetic blockade OR peripheral blocks OR surgery) AND (child OR adolescent OR pediatric). Initial search results were limited to English and Spanish language articles. The references in the selected articles were used to identify additional relevant sources. In addition, the authors identified a limited number of articles or chapters from personal readings.

31 studies met the criteria to be included in this review *(Table 1)*. Their full texts were analyzed for retrieving information as: the invasive treatment used - including prior and concurrent conservative interventions-, outcomes measured, type of study, patient characteristics, quality of the study, design and methodology.

3. Review of the evidence

Conservative management:

Although reviewing CRPS non-invasive therapy is not the goal of this article, we have considered it appropriate to briefly describe the most accepted model of management for this condition. CRPS in childhood and adolescents seems to respond favorably to conservative multimodal inpatient therapy (Maillard et al. 2004, Katholi et al. 2014). In the largest pediatric trial reported to date, 92% of children and adolescents were free of symptoms after an intensive physical therapy program (Sherry et al. 1999). Other smaller series identified in the literature have presented recovery rates of 70% as well after applying conservative management (Stanton-Hicks et al. 1998, Low, Ward, and Wines 2007b, Kachko et al. 2008), however recovery or resolution is not always well-defined.

Nonetheless the long-term prognosis is unclear and between 28% and 48% of patients with pediatric CRPS experience a relapse (Stanton-Hicks et al. 1998, Sherry et al. 1999, Wilder 2006, Low, Ward, and Wines 2007b, Tan et al. 2008). Consolidation of the evidence suggests that conservative treatment of pediatric CRPS should form the basis of first-line treatment. Being the medication, the psychological and the physical therapies clearly the core of the initial treatment. However, further interventions are needed when the condition does not resolve or a relapse occurs.

Invasive Pain Therapy

The relevance of invasive therapies in children who otherwise do not respond to conservative management or medications after a few weeks of treatment has not been established in pediatric patients (Nordmann et al. 2006, Rodríguez et al. 2007, Olsson et al. 2012, Zernikow et al. 2015). There is not a single randomized control trial to date comparing the conservative and the invasive management of this particular group of patients. The largest series of pediatric cases showed that between 29% to 35% of children with CRPS needed interventional measures to manage this condition successfully (Kesler et al. 1988, Lee et al. 2002, Kachko et al. 2008).

Within this review we have identified 31 publications published between 1980 and 2015. Most studies were case series and case reports (n=28), including a total of 108 patients. One randomized control trial of 23 patients and two controlled studies of 40 patients in total complete the collection of studies of this review *(Table 1)*. The entire collection of publications contained data of 171 patients. The characteristic of the population who received invasive procedures correlates with the characteristic of the children showed by other publications affected by this syndrome who do not receive this sort of treatments *(Table 2)* (Tan et al. 2008, Logan et al. 2013). Spontaneous pain and functional disability were the two outcomes measured with more assiduity. The overall improvement for spontaneous pain was documented in 79% of cases, 16% of patients showed no change. Functional disability was reported in 25 publications, 24 of them showed improvement after treatment.

This study reveals that the most used procedure was the sympathetic blockade *(Table 1)*. Singular or continuous sympathetic blocks were used in 15 studies, 123 patients. Within this group of studies we found the only randomized control trial (Meier et al. 2009) and two controlled studies (Greipp et al. 1988, Dadure and Capdevila 2005). Numerous types of blocks are included in this group, for example: the sympathetic blocks of the ganglion *stellatum* for CRPS in the arm, the block of the lumbar *truncus sympathicus* for CRPS in the leg or the thoracic block of the Kuntz´s nerve. Local anesthetic blockade of the sympathetic chain has been widely used to treat CRPS in adults, however the empirical data is confusing yet (Ramsaroop et al. 2001, de Oliveira Rocha et al. 2014). A systematic review revealed the paucity of published evidence to support the use of local anesthetic sympathetic blockade as the 'gold standard' treatment for CRPS (Cepeda et al. 2005, Martin et al. 2013, Stanton et al. 2013). Likewise, we can concl that its efficacy has not been proven for the treatment of CRPS in adults. The data in children is far scarcer and uncertain, that is why this treatment has been relegated to a more tentative choice in pediatric CRPS. Additionally, most of the publications analyzed revealed that multiple invasive procedures were needed during the period of treatment with this technique, increasing the risks of side effects (Stanton et al. 1993, Kachko et al. 2008).

The spinal drug infusion of local anesthetics was used in 11 studies, all of them in the last 15 years. Spinal drug infusion through epidural catheter has been largely used in this group of patients when the physiotherapy program needs to be supported or when the symptoms do not decrease with conservative management. Epidural drug infusion with local analgesics is a viable alternative when conventional treatment do not achieve acceptable results, it also has the advantage of the supplementation with opiates to the local anesthetics to offer better analgesia. The complications and risks of this technique (eg. respiratory depression, motor block, sympathetic block resulting in hypotension and urinary retention) can be avoided by careful titration of the infused medications and adequate patient and family education. To date there is no randomized trial for spinal drug infusion in CRPS, however there are numerous reports

supporting this technique. Of 37 adult CRPS patients treated with continuous epidural infusion of bupivacaine and fentanyl, nearly 90% had a reduction in their symptoms when treated within 12 months after onset. However, the success rate diminished considerably when treatment was began more than a year after onset (Moufawad et al. 2002). In the pediatric literature reports are fewer yet analogous to those found in adults which would suggest a favorable outcome (Farid and Heiner 2007, Rodríguez et al. 2007, Kachko et al. 2008, Saito et al. 2015). Some authors highlight the importance of avoiding delay for treating CRPS invasively(Rodríguez et al. 2007, Saito et al. 2015). Therefore, we conclude that early treatment with continuous epidural anesthesia may be promising when initial non-invasive management is ineffective.

SCS has demonstrated efficacy in CRPS type 1 in adults (Grabow et al. 2003, Tracy Cameron 2004, Kemler, de Vet, et al. 2008). SCS in adults, same as in pediatrics, an electrode is placed in the epidural space on the dorsal aspect of the spinal cord at the level of the nerve roots innervating the painful area. Electrical current from the electrode brings about paresthesia, a sensation that suppresses the pain. This technique has become more popular during the last decade for the management of CRPS in adults, obtaining successful results in most cases (Tracy Cameron 2004, Turner et al. 2004, Kemler, De Vet, et al. 2008, Taylor et al. 2012). In the pediatric population it has been suggested as a possible option when the subject is resistant to all conventional treatments (Turner et al. 2004, Bennett and Brookoff 2006, Grabow et al. 2006, Kemler, de Vet, et al. 2008), but only a few examples of successfully treated CRPS in children have been presented to date, 3 case series with 11 people in total (Wilder 2006, Rodríguez et al. 2007, Olsson et al. 2012). Therefore, to the best of our knowledge SCS can be a useful and promising treatment for CRPS in pediatric patients who do not respond to conventional treatment. Nevertheless, due to the small and non-controlled design of these case series, further studies are needed to verify that SCS can be recommended for its use in this group of patients.

There are others invasive techniques that have been considered when conventional therapy has failed in pediatric CRPS. Regional anesthesia has been tried in 36 patients during the last years, mostly during the last 10 years, however the results do not appear to be as good as with some of the techniques mentioned previously. Similarly, intravenous regional blocks with lidocaine counts with unsatisfactory or unclear reports in general, being the decrease in spontaneous pain and functional disability improvement less than with any other procedure, 55% and 50% respectively.

Table 2.

Characteristic	Adult [1]	**Paediatric** [2]
Age*	45	12.8
Gender ratio	Male predominance	Female predominance (85%)
Extremity affected	Upper	Lower (80%)
Trauma	Mild- Severe	Minor- Mild
Limb temperature	30% cooler	70% cooler
Edema	40%	75%
Prognosis	Variable, long term disability	Excellent recovery in most cases
Relapse rate	10%	30%

Table 2.- Adult vs. Paediatric CRPS characteristics.

* mean age at presentation of the symptoms. [1]Data extracted from CRPS adult literature. [2] Description of patients comprised in this review.

Table 3.

Reference	Year	Intervention	n	Outcome measure.[1]	Length[2]	Previous medication[3]	Adverse effects	Improvement (% *patients)*	Comments
Rodriguez et al. (33)	2015	LA Spinal inf. SCS	10 (6) *	Yes	52 w.	Opioids (67%) NSAIDs (83%) Anticonvul (100%) Antidepress (67%) Capsaicin (100%)	No	100%	This study showed successful results after applying a multimodal and progressive approach including invasive measures as well as physical management and novel medication as the capsaicin 8%patch.
Olsson et al. (Olsson et al. 2012)	2012	SCS	7	Yes	52/250w	Opioids NSAIDs Anticonvul Antidepress Ketamine (14%) Epidural L.A (28%)	Yes, Local infection	Full recovery (72%) Minor symptoms or recurrences (28%)	Olsson´s study comprised seven girls, presenting with severe, incapacitating and therapy-resistant CRPS-I, who were subjected to SCS. Good technique description but poor methodology.
Meier et al. (Meier et al. 2009)	2009	Cont Lumbar sympath block Lidocaine iv	23	Yes	-	”6-week trial of aggressive physical, bio-behavioral, and pharmacological therapies,”	Minor	LSB: Complete (29%). Adequate (41%) Minimal (32%) Lido iv: Minimal (84%) Adequate	The purpose of this study is to compare the efficacy of lidocaine administered by lumbar sympathetic to IV route. Excellent methodology and clear results. No

								(16%)	follow up period.
Kachko et al. (Kachko et al. 2008)	2008	Epid cath (1) Stellate gang block (1) Regional anesth .(2)	14 (4) *	Poorly	8w.	NSAIDs Anticonvulsive Antidepressant	.	Full (78%) Partial (15%) Recurrence (29%)	Retrospective study that aimed to assess the efficiency of the multimodal management of CRPS. Limited but illustrative of the actual clinical set up of many Pain Treatment Units.
Stanton et al. (Stanton et al. 1993)	1993	Sympath block Regional anesth	36 (x) *	Poor	-	NSAIDs Anticonvulsive Antidepressant Opioids	-	Moderate or poor	Review of the experience at this center. They aimed to present diagnostic criteria for pediatric CRPS. Management and outcomes poorly described.
Wilder et al. (Wilder et al. 1992)	1992	Sympath block	70 (37)	Yes	20w	NSAIDs Antidepressant	-	Full (71%) Moderate (13%)	Wilder retrospective study reported his experience with a multimodal treatment, using in more than 50% invasive techniques.

Table 3. Relevant publications, selection by the authors.

(1) Outcome measure carefully described. (2) Length of the follow up -weeks-. (3) Medication prior invasive treatment. * Number of patients treated with invasive measures within the total of patients.

3. Discussion

Complex Regional Pain Syndrome is characterized by complex clinical presentations and a pathophysiology that seems to be multifactorial in nature, characterized by an aberrant host response to tissue damage (Meier et al. 2006, Gierthmühlen et al. 2011, Marinus et al. 2011). Most of the clinical features of this condition can be explained by the confluence of three major pathophysiological pathways: vasomotor dysfunction, aberrant inflammatory mechanisms, and maladaptive neuroplasticity. The clinical heterogeneity of the disorder is indicative of the inter-individual variability in the activation of these pathways after tissue injury (Jänig 2009, Coderre and Bennett 2010, Marinus et al. 2011).

The recommendations of the Special interest group in Neuropathic Pain (NeupSIG) of the IASP for the pharmacological management of neuropathic pain (NP) only considered treatments with at least 2 high-quality randomized clinical trials (RCTs) (Dworkin et al. 2010). Nonetheless, there is limited evidence evaluating interventional treatments for NP, and many interventions used in clinical practice to manage NP in refractory patients are supported by weak, if any, evidence (Dworkin et al. 2013). This evidence is even more fragile when talking about the management of CRPS, and completely exiguous when referring to the management of pediatric CRPS.

Nonetheless, the scientific consensus is that the cornerstone of the CRPS management should be the restoration of function. Acknowledged therapies include a combination of pharmacotherapy, physical therapies and psychotherapy where appropriate (Lee et al. 2002, Zernikow et al. 2012, Chopra and Cooper 2013, Borchers and Gershwin 2014). Several studies highlight that early mobilization of the affected limb assisted with cognitive behavioral techniques is the most important part of the management process in children (Lee et al. 2002, Wilder 2006). In our experience this is highly important but so is the use of medication and the early diagnosis of the disorder, which substantially influence the prognosis of the condition (Murray et al. 2000, Lee et al. 2002, Berde and Lebel 2005, Finniss et al. 2006). Low et al. showed that children who received a prompt diagnosis (less than 12 weeks), and therefore were offered treatment more rapidly achieved a quicker and more successful remission of CRPS when compared to those whose diagnosis was delayed (10.6 and 21.5 weeks)(Low, Ward, and Wines 2007b).

Unfortunately a significant percentage of children who suffered CRPS do not respond to conservative treatments. Only those patients who do not improve successfully after being treated with a complete pain management plan during a reasonable time are candidates for invasive pain therapies (Rodríguez et al. 2007). Unfortunately the evidence supporting the use of these procedures is weak. This review shows that the methodological quality of the existing data is low as most of the publications found are case series or case reports representing level IV evidence. On top of that a very low percentage of publications used the established diagnostic criteria for CRPS of the IASP. Additional negatives aspects of this group of publications are that validated outcomes tools were not used in most cases and that the follow up period were usually not reported or rather too short.

Within the invasive techniques described in these publications, we must highlight the continuous epidural infusion and the SCS. They seems to have an important effectiveness and to be minimally invasive and reversible, besides in adults they have been shown to be very effective for certain forms of NP (Moufawad et al. 2002, Linderoth and Meyerson 2010, Dworkin et al. 2013). Olsson et al. concluded that the SCS was

successful for treating CRPS in all their pediatric patients (Olsson et al. 2012), however this conclusion can be questionable from our point of view as in one of the patients the symptoms ceased after the patient had not responded well to any stimulus of the SCS and other patient of the same series developed an infection which seriously compromised the treatment. Rodriguez et al. had a great experience with the SCS, abolishing the symptoms in 3 children with a well-defined history of uncontrollable CRPS (Rodríguez et al. 2007). This study together with the positive experience of Wilder et al. (Wilder 2006) encourage the need for a better understanding and use of SCS in CRPS. Likewise, the use of epidural catheter for the infusion of local anesthetics has been implemented deeply in the last years. The majority of publications agreed that the treatment importantly diminish the pain and improve functionality of the limb affected. Regrettably, very few of these publications described the process (the space where the catheter was implanted, the concentration, the dose, etc.), the outcome or the side effects if any.

Side effects were infrequently reported. Infections only occurred in two patients and minor side effects were reported only in 10 studies. 16 of 83 reported cases experienced a relapse. From our perspective, based in our experience and the literature behind these procedures, we believe that the side effects in this collection are underreported.

4. Recommendation

Based upon the available evidence with regard to effect and complications, we recommend the following algorithm for the management of pediatric CRPS *(Figure 1)*.

A crucial first step for the management of this condition appropriately consists in making an accurate and early diagnosis. We strongly encourage basing the diagnosis in the CRPS criteria of the IASP (Harden et al. 2007), despite this set of criteria has been made for adults. The real goal of the physician must be the restoration of the normal function of the affected limb, using every possible management tool to achieve this. Initially, physical therapy, psychological support and adequate pharmacological treatment should be used together, complementing one another and aiming to make the condition resolve within a few weeks. Pharmacological measures are prescribed on a symptom-oriented basis. However, new approaches should be adopted when fitting within a mechanism-based management (Gierthmühlen et al. 2014). Analgesics, anti-inflammatory therapy, antidepressant and antiepileptic drugs have been used to date. However, new topical drugs such as the high-concentration capsaicin patch have been tried within the past few years with excellent results (Rodríguez et al. 2007).

Based on our experience (Rodríguez et al. 2007), the heterogeneity found at the literature regarding the duration of the conservative management for treating CRPS together with the lack of knowledge of its precise pathophysiology, we recommend that after a reasonable time of 4 to 5 weeks under intensive multimodal therapy without successful results, more invasive options should be considered. Before failure of conservative management is taken as a reason to contemplate invasive measures as the following step, only high-quality conservative treatment should be implemented. Therefore, knowledge concerning such treatment needs to be increased. Patients with CRPS with severe pain, allodynia, or with a measurable skin temperature difference compared to the non-affected limb that do not respond to the multi-modal conservative management should be put forward for therapies such as spinal infusion of drugs,

sympathetic blockades or SCS. In our opinion, after reviewing the literature on the topic, the initial option for children who do not respond successfully to conservative management is the continuous epidural

Figure 1. Clinical algorithm for the management of pediatric CRPS.

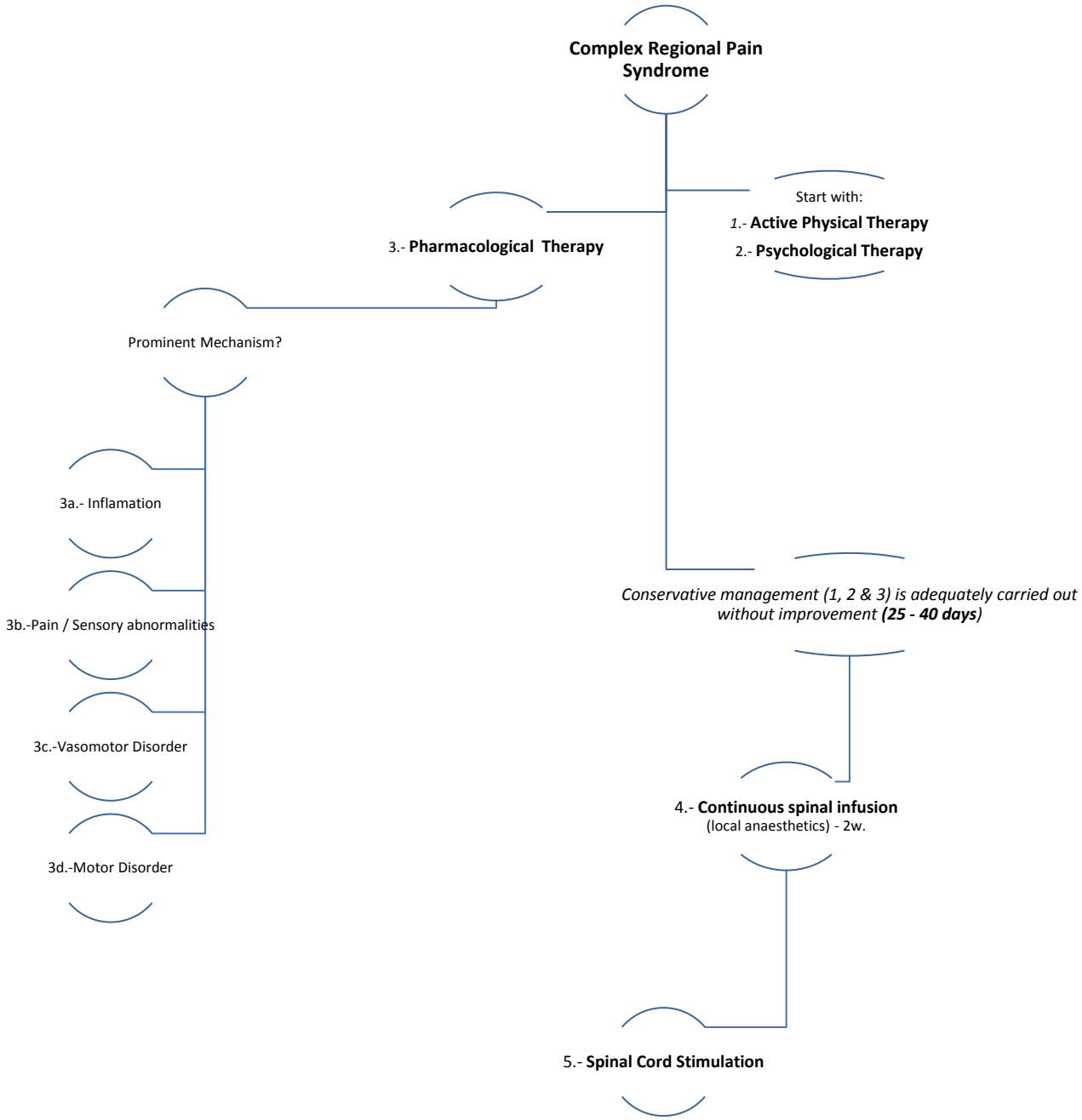

Figure 1. The first crucial step is the correct and early diagnosis. Immediately after, a multimodal approach must be taken. Active physical therapy, psychological support and the correct pharmacological approach must be planned. Initially a sign and symptom oriented pharmacological treatment is engaged. Patients who do not respond succesfully to the conservative management carried out during a period of 25 to 40

days will attempt with invasive techniques. First LA continuous epidural infusion for 2 weeks and then SCS can be recommended after multidisciplinary evaluation.

infusion of local anesthetics (Rodríguez et al. 2007). This technique provides analgesia and sympathetic blockade throughout the time the catheter is attached. This is usually enough to control the condition and prevent the reappearance of symptoms (Rodríguez et al. 2007). We recommend its use for 10 to 14 days. Having the catheter more time could increase the risk of infection (Sethna et al. 2010) or further side effects secondary to the drug infusion or the catheter itself. However, this technique can fail or the symptoms can reemerge after the catheter is removed. In this case, SCS can be recommended after multidisciplinary evaluation and a successful trial stimulation. SCS is a minimally invasive and reversible technique that facilitates physical therapy and helps decrease medication (Olsson et al. 2012).

6. Conclusion

This article proposes a multidisciplinary management approach to the treatment of CRPS in children for whom the standard treatment has not been successful. Because of the severity and rapid progression of the symptoms in CRPS we consider that an early diagnosis of the condition together with comprehensive, and individualized multidisciplinary treatment offers children with CRPS the best opportunity for a complete recovery. Within this approach we encourage clinicians to consider invasive procedures as a reliable option of treatment. Unfortunately the type of technique that should be applied when high quality multimodal conservative treatment fails cannot yet be based on empirical data. Therefore, since the significant limitations of the evidence, interventional treatments for the management of CRPS in children should ideally be offered in clinical and research settings with experience and ability to understand and report the outcomes. This will make it possible to substantially improve the evidence on which forthcoming recommendations are established.

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THESIS CONCLUSIONS

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Analysis in detail of the results obtained, we have drawn up the following conclusions:

This work travel among different areas within one of the most disabling forms of chronic pain, Neuropathic Pain. NP following peripheral nerve injury is associated with hyperexcitability in damaged myelinated sensory axons. NP continues being fairly difficult to diagnose and treat nowadays as we do not fully understand the molecular mechanisms that cause it.

After a sensory nerve is cut or otherwise damaged it becomes hyperactive and produces spontaneous electrical activity that the brain interprets as nociceptive signals. However, it is not fully understood how cutting a nerve affects the ion channels in a way that generates this hyperactivity. Different types of ion channel are found in different regions of the nerve cell. At the first part of this work we have investigated the composition and distribution of shaker-type-potassium channels (Kv1 channels) within the nodal complex of myelinated axons following injury. Soon after nerve damage occurred, at the neuroma that forms after damage, expression of Kv1.1 and 1.2 was markedly decreased. In contrast Kv1.4 and 1.6, which were hardly detectable in the naive state, showed increased expression within juxtaparanodes and paranodes following injury, both in rats and humans. At the same time, electrical activity in the cut nerve increased, and the recovering animals responded in ways that suggested they were hypersensitive to the nerve being touched.

Three weeks after the injury, most rats lost their hypersensitivity and the electrical activity in the cut nerve returned to near-normal levels. It was also found that the recovering nerves contained new subtypes of type 1 potassium channels. These potassium channels did not just appear in the juxtaparanode: they also invaded the ‘fence’ region that normally separates potassium channels from sodium channels. The same was observed to happen in the nerves of patients that suffer from NP due to a nerve injury.

At this late time point after nerve injury, blocking the activity of potassium channels produced the same abnormal increase in the nerve’s electrical activity as seen immediately after the nerve had been cut. The rats’ hypersensitivity to touch also returned. This suggests that changes in the molecular composition and distribution of axonal Kv1 channels, therefore represents a protective mechanism to suppress the hyperexcitability of myelinated sensory axons that follows nerve injury.

At the second stage of this thesis we moved to the diagnostic area of NP. Here we investigated how in human studies the screening tools for diagnosing NP are able allocates patients into sensory profiles. More precisely, we analyse if the overall PDQ score or its items reflect phenotypes of sensory loss in NP as determined by QST.

The results obtained shows that the PDQ score is not sensitive enough to distinguish different types of sensory loss in patients with NP. While our results demonstrate that there is variability in the responses to four items between groups of people with various kind of QST-detected loss of sensation, they do not provide clear information on an individual patient basis. Therefore, we can conclude that PDQ is unable to clearly separate between patients with varying types of loss of sensation while loss of function can be detected for all sensory qualities in QST. Nevertheless, more complex analysis, identifying items that differentiate between types of sensory loss in a new battery of questions would be an interesting approach for a future study. Besides, enquiring about autonomic disorders, which commonly appear in patients with small fibre neuropathy would be helpful for the development of this and other specialized questionnaires in the detection of NP phenotypes. This may improve clinical practice in the treatment of NP outside specialized centres, as, at present, none of such screening tools are sensitive enough to document sensory loss.

Finally, the third, and last part of this work focused its attention in the management of NP. Because NP does not only appear during adulthood, I decided to dedicate part of this study to NP in children, particularly to the invasive management of CRPS during childhood. Within this section we examined and analysed the data data currently existing in this issue. Furthermore, we suggest a management algorithm based in the evidence reviewed and our team experience, which has been also described and analyse as part of this thesis.

The conclusion of our review is that invasive techniques have been used to treat CRPS over the last few decades; however, the evidence for their use is still very weak. Therefore, invasive management should be contemplated only when high standard conservative management has failed to work.

Based on the existing literature and in our experience, described in chapter 3, we propose a multidisciplinary management approach to the treatment of CRPS in children for whom the standard treatment has not been successful. Because of the severity and rapid progression of the symptoms in CRPS, we recommend that an early diagnosis of the condition together with comprehensive and individualized multidisciplinary treatment offers children with CRPS the best opportunity for a complete recovery.

RESUMEN EN ESPAÑOL

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El dolor crónico (CHP) es uno de los problemas de salud más onerosos que enfrenta la sociedad actual; Su costo para los países occidentales es casi el mismo que para el costo del cáncer y la diabetes mellitus combinados (Vos et al., 2012). La investigación epidemiológica confiable sobre la PP proporciona información clave sobre la prevalencia y los factores relacionados con su génesis y perpetuación. Mejorar nuestra comprensión de los detalles que rodean la enfermedad mejorará el manejo clínico, limitando la gravedad y minimizando la discapacidad. Existen hipótesis fundadas de que las estimaciones recientes de la carga global de la enfermedad han subestimado la contribución de ChP (Smith et al., 2007, Shmagel et al., 2016). La Organización Mundial de la Salud (OMS) y otros investigadores pronostican que para el 2030 los cuatro principales responsables de la carga global de la enfermedad serán las enfermedades coronarias, los accidentes de tráfico, la depresión y las enfermedades cerebrovasculares (Vos et al 2015). Como era de esperar, el ChP es una comorbilidad importante vinculada a todos ellos; Sin embargo, ChP es mucho más que una simple comorbilidad de otra lesión o enfermedad. ChP se reconoce hasta la fecha como una condición por derecho propio (Merskey 1994, Tracey y Bushnell 2009, Treede et al., 2015). Aproximadamente una quinta parte de la población europea adulta sufre de ChP (Breivik et al., 2006) y, además de la carga física y emocional que esto trae, el costo monetario para la sociedad es enorme, estimado actualmente más de 200.000 millones de euros por año en Europa y mas de 150 millones por año en los Estados Unidos (Tracey and Bushnell 2009).

Nuestra comprensión de la fisiopatología de ChP ha aumentado sustancialmente en los últimos 20 años, incluyendo de la periferia al cerebro (Dubin y Patapoutian 2010, Baron et al., 2012). No obstante, todavía no entendemos por qué el CHP se desarrolla en algunas personas y no en otras, pero sabemos que la magnitud o el tipo de lesión, las características psicosociales, las creencias religiosas, la ocupación, el nivel educativo, la raza o el código postal no son predictores confiables. 2010). La investigación extensa en la genética de ChP también no ha podido predecir el inicio de la enfermedad, posiblemente debido a la gran cantidad de genes implicados ya los resultados contradictorios de la investigación (Diatchenko y otros 2007, Møller y Jensen 2010). El tratamiento de ChP es generalmente una tarea tediosa y compleja, donde el especialista del dolor y el paciente deben crear una relación fuerte y genuina. Parece que, a pesar del gran progreso en múltiples campos, poco se ha hecho (White et al., 2011, Rajapakse et al., 2014, Moulin et al., 2015).

Dentro de la imagen evolutiva la activación de cualquier tipo de nociceptor especializados como el alto umbral mecanorreceptores tienen un papel protector, actuando como un sistema de advertencia para las señales amenazantes. Sin embargo, mientras que el dolor inflamatorio es adaptativo, la evolución no ha tenido éxito en explicar nuestra mayor capacidad para sobrevivir a enfermedades, traumatismos o agresiones iatrogénicas destinadas a extender o mejorar la calidad de vida. En tales ambientes, el dolor ya no es una característica útil, sino que se convierte en la enfermedad misma. Sin embargo, es natural pensar en el dolor como una entidad homogénea, esto es demasiado simplista. De hecho, existen diferentes tipos de dolor, cada uno con diversos mecanismos fisiopatológicos y neurobiológicos. La clasificación más reconocida divide el dolor en dos tipos principales: dolor nociceptivo y neuropático; Sin embargo esta clasificación es otra vez más simplista. Por lo tanto, recientemente la nueva categoría de DCI para "Dolor crónico" trató de incluir y dividir los trastornos clínicamente más relevantes, los cuales se dividieron en 7 grupos: dolor crónico primario, dolor crónico de cáncer, crónica crónica (5) dolor de cabeza

crónico y dolor orofacial, (6) dolor visceral crónico y (7) dolor crónico osteomuscular, esta división es importante porque no sólo indica la etología y la neurobiología Sino también el tratamiento puntual El dolor nociceptivo puede clasificarse como visceral o somático (Treede et al., 2015).

El dolor neuropático se define como el dolor resultante de una lesión o disfunción del sistema somatosensorial (Treede et al., 2008), por lo que el daño tisular afecta directamente al sistema nervioso, dando lugar a la generación de descargas ectópicas que puentean la transducción Et al., 2010). NP es ampliamente identificado como uno de los síndromes de dolor más complicados de manejar, y los resultados son a menudo decepcionantes. Esto se debe en parte a que la contribución de la neuropatía al dolor que se presenta en la atención primaria puede no ser reconocida (Rodríguez et al., 2007, Leadley et al., 2012) y hay evidencia de un uso de fármacos subóptimo en el tratamiento de NP (Smith et al., 2007, Jongen et al., 2013, Helfert et al., 2014). La investigación epidemiológica en el campo de NP es problemática y las razones son diversas: la falta de una definición de caso legítimo que realmente indique la (s) condición (es) bajo consideración y que sea factible utilizar en estudios poblacionales; La calidad heterogénea de los estudios, el uso de medios inconsistentes de verificación de casos y los criterios de inclusión o exclusión en los que el dolor no es la principal queja (Torrance et al. 2006, Smith, Macfarlane, et al. 2007, Smith et al. 2012, van Hecke et al. 2013). Los cálculos existentes de la prevalencia general de la población difieren ampliamente, y es plausible que la gente experimente síntomas neuropáticos en mayor medida de lo que se ha diagnosticado con una afección neuropática relacionada con el dolor (Torrance et al 2006, Freynhagen y Baron 2009). Los estudios epidemiológicos de NP son de gran valor para decidir las necesidades de recursos (educativas, económicas y clínicas) en la atención primaria y en los centros hospitalarios, e informar sobre las estrategias de prevención y la orientación de la gestión. A medida que las herramientas de cribado validadas para identificar NP se han desarrollado (Torrance et al., 2006, Bennett et al., 2007, Haanpää et al., 2011, Mick y otros, 2014), estos han permitido estudios epidemiológicos basados en cuestionarios y hay una creciente Cuerpo de literatura que investiga la epidemiología de NP síntomas y condiciones en la sociedad. Hasta la fecha, la estimación más fina de la prevalencia de la población de dolor con características neuropáticas es probable que se sitúe entre 7% y 10% (van Hecke et al., 2014).

NP se caracteriza por síntomas positivos y negativos que abarcan dolor, hipoestesia al tacto, hormigueo, descargas eléctricas y pasadores y agujas (Woolf y Mannion 1999). Varios estímulos nerviosos perjudiciales en el sistema nervioso central o periférico pueden conducir a NP, aunque las características clínicas del dolor pueden ser similares a través de los diferentes síndromes neuropáticos y etiologías (Freeman et al., 2013). Aunque muchas formas de dolor nociceptivo, y algunas formas de dolor neuropático, pueden conferir beneficios evolutivos, NP crónica es en todo momento maladaptativa. Los enfermos de NP manifiestan regularmente percepciones sensoriales paradójicas acompañadas de dolor como un síntoma primordial positivo combinado con sensaciones degradadas inducidas y degradadas. Estas sensaciones son típicamente muy únicas y no se han sentido previamente por los pacientes. Esta combinación de signos de hiposensibilidad e hipersensibilidad no es inusual en trastornos neurológicos; Por ejemplo, cuando aparece espasticidad después de la lesión de la médula espinal o cuando se desarrolla temblor parkinsoniano después de la degradación de la sustancia negra. Sin embargo, comparado con estas interrupciones motrices, el dolor como síntoma sensorial subjetivo es difícil de medir y abarca no sólo características físicas, sino también atributos psicológicos y emocionales (Gustin et al. 2015, Thacker 2015). Las anomalías sensoriales distintivas son hallazgos clave para diagnosticar y

clasificar NP apropiadamente, y para diferenciar esto de otros patrones de dolor. Los principales retos en el desarrollo de un enfoque holístico orientado a la gestión de PN abarcan un diagnóstico adecuado de la etiología y los mecanismos por debajo, el reconocimiento del tipo de dolor y la evaluación de su patrón y su perfil sensorial y la determinación del tratamiento adecuado.

Dolor Neuropático (DN) abarca, o acompaña, a una amplia variedad de patologías ligadas con enfermedad o lesión del sistema somatosensorial a nivel central o periférico, oscilando su prevalecía en la población general en torno al 7-10%. En la actualidad la mayor parte del conocimiento que tenemos de los mecanismos subyacentes al origen y desarrollo del DN han sido obtenidos de estudios en ciencias básicas. Los obvios problemas que surgen de la traslación en investigación médica revelan las importantes limitaciones de ciertos modelos experimentales, y desde esa línea se originan esenciales limitaciones para la investigación clínica. A pesar de estas dificultades, la comunidad científica escruta sin cesar nuevas y mejores ideas que nos ayuden a entender mejor la patofisiología del dolor basándose fundamentalmente en nuevos descubrimientos en el campo de las ciencias básicas.

Hasta la fecha, tanto los datos básicos como los humanos sugieren que una lesión de vías aferentes es necesaria para el desarrollo de NP. Además, numerosos estudios demuestran que no uno sino varios mecanismos pueden inducir a NP. Significativamente, un gran número de estos mecanismos no se basan en la causa de la enfermedad, el mismo mecanismo puede ser detectado en diferentes condiciones. En un solo paciente, varios mecanismos pueden estar implicados y diversos mecanismos podrían ser el origen del mismo síntoma. Esto no sólo evidencia la complejidad de NP, sino que también subraya la importancia clínica de identificar mecanismos de dolor subyacentes en cada paciente. Debido a que se requieren diferentes planes de manejo para diferentes mecanismos de dolor, un enfoque de tratamiento basado en mecanismos puede guiar a los médicos para obtener mejores resultados.

Uno de los principales problemas en el manejo de estos síndromes es el hecho de que los tratamientos se aplican de manera uniforme cualquiera que sea el cuadro clínico, mientras que estos estados neuropáticos son de hecho muy heterogéneos. En los últimos años se han realizado importantes avances clínicos en este campo, tras la validación de nuevas herramientas clínicas y la estandarización de paradigmas de perfil sensorial, permiten mejorar la caracterización clínica de estas condiciones. Numerosos estudios han demostrado que la NP es una entidad clínica consistente, pero es multidimensional en cuanto a su expresión clínica, con perfiles sensoriales disímiles, potencialmente indicativos de mecanismos fisiopatológicos particulares. Este nuevo concepto de NP debe perfeccionar la caracterización de los perfiles de los pacientes en los ensayos clínicos y poner a disposición los datos esenciales para el desarrollo de nuevos y clínicamente más sólidos enfoques traslacionales.

Una ruta para avanzar en esta etapa en los contextos clínicos de investigación es la hipótesis de que los mecanismos del dolor se pueden deducir mediante el estudio de los síntomas individuales y patrones de signos con los métodos mencionados anteriormente. Al estudiar el efecto del tratamiento que se dirige a estos mecanismos propuestos, el concepto de tratamiento basado en mecanismos puede ser validado. Este enfoque permitirá diseñar estudios clínicos más centrados en un mecanismo de síntomas relacionados y el tratamiento de los signos en lugar de los ensayos basados en la etiología. Hasta el momento, los datos actuales contribuyen a comprender los vínculos entre al menos algunos síntomas clínicos y los mecanismos subyacentes sugeridos, sin embargo, todavía hay un largo camino para caminar en este campo. La segunda parte de esta tesis pretende establecer o aclarar algunos vínculos entre la TVQ y los síntomas clínicos manifestados por los pacientes con NP.

NP es también un tema importante durante la infancia y durante la adolescencia, sin embargo, muchas de las etiologías subyacentes en las que ocurre son infrecuentes o nunca se encuentran en niños. Sin embargo, se han descrito hasta la fecha varias causas de NP, incluyendo lesión traumática, enfermedad neurológica y metabólica, disfunción hereditaria del nervio sensorial y CRPS. La evidencia en estudios básicos y clínicos de NP ha revelado diferencias significativas relacionadas con la edad en la presentación clínica y el pronóstico. Clínicamente, el diagnóstico, la evaluación y el tratamiento de NP en niños se basan en métodos, experiencia y evidencia adquirida a partir de datos en adultos. Por lo tanto, y debido a la complejidad cuando se establecen ensayos clínicos con niños, es especialmente importante aumentar los datos disponibles en los niños compartiendo todas las experiencias que pueden ayudar a los médicos a mejorar el conocimiento cuando manejan estados neuropáticos durante la infancia. La tercera y última parte de este trabajo, centra su atención en la gestión de NP en los niños; Particularmente en el manejo invasivo de la CRPS.

El tratamiento de la NP sigue siendo un gran desafío, porque un gran número de pacientes no experimentan suficiente alivio del dolor, como se deduce de los resultados de los ensayos clínicos y de la experiencia clínica. Esta complejidad en el tratamiento podría ser consecuencia de la heterogeneidad de los mecanismos fisiopatológicos de NP y las facetas emocional y psicológica comúnmente coexistentes de ChP. En primer lugar, un diagnóstico riguroso puede revelar la fuente del dolor. Por lo tanto, un adecuado manejo del mismo puede resultar en alivio parcial o total del dolor. Cuando se comienza el tratamiento sintomático, la educación de los pacientes, la inclusión de información sobre NP, el plan de manejo y los efectos secundarios factibles de cualquier tratamiento -farmacológico o no farmacológico- son indispensables para aumentar el cumplimiento por parte del paciente. Para evitar las expectativas poco realistas de los enfermos de NP sobre la tolerabilidad y la eficacia, los objetivos de gestión realistas deben ser determinados. El agotamiento del dolor de por lo menos el 25% es generalmente bienvenido para ser un resultado clínicamente significativo (Attal 2011). Además del dolor, tanto la calidad de vida relacionada con la salud como la alteración del sueño deben evaluarse al evaluar la eficacia analgésica (Smith et al., 2007, Gustorff et al., 2008).

Además, la ansiedad y la depresión coexistentes pueden obstaculizar el manejo exitoso del dolor y deben ser reconocidas y dirigidas para un tratamiento específico (Maletic y Raison 2009, Brod et al., 2014). En contextos clínicos, esta complejidad se tiene en cuenta mediante un enfoque terapéutico multimodal, que incluye opciones de manejo farmacológico y no farmacológico, tales como terapia física, intervenciones psicológicas como conducta cognitiva y terapia ocupacional (Kerns et al., 2010, Kozma et al 2014, Casanova-García et al., 2015). A pesar de que la eficacia de este punto de vista biopsicosocial multimodal se ha informado rutinariamente en condiciones de ChP distintas de NP, su fortaleza en pacientes con NP es bien aceptada (Garven et al., 2011). En aquellos que desarrollan dolor de miembro fantasma y CRPS, se ha demostrado que los tratamientos no farmacológicos como la terapia cognitivo-conductual y la terapia ocupacional, así como los nuevos métodos, como la imagen de motor graduada (incluida la terapia de espejo), reducen el dolor y mejoran la funcionalidad (Katholi Et al., 2014, Simons 2016). Del mismo modo, los tratamientos invasivos han sido reportados como métodos útiles en estados neuropáticos cuando se usan adecuadamente (Slavin 2008, Pereira y Aziz 2014).

Terapia basada en el mecanismo. Las condiciones de ChP son estados heterogéneos con un gran número de características genéticas, fisiopatológicas y psicosociales, las cuales son caprichosamente expresadas en cada persona. Como resultado, los enfoques científicos y clínicos actuales deben estudiar

los diferentes elementos subyacentes lo más detallados posible, con el objetivo de adaptar la gestión al paciente único, una estrategia que se denomina medicamento para el dolor personalizado. NP se sugirió en el que los síndromes de dolor se agrupan sobre la base de los mecanismos fisiopatológicos subyacentes de la causación del dolor en lugar de en la causa de la enfermedad (Woolf et al., 1998). La idea que apoyaba esto era que podría ser factible y más apropiado conducir tratamientos específicos hacia condiciones específicas que miran su procesamiento nociceptivo particular.

La comprensión actual sobre los mecanismos del dolor ha crecido excepcionalmente en los últimos años, quedando claro que numerosos mecanismos tanto en el sistema nervioso central o periférico actúan solos o en combinación en una sola persona. Como una condición necesaria para transferir la idea de tratamiento basado en mecanismos a la práctica clínica, es esencial desarrollar instrumentos de diagnóstico que contribuyan a determinar los mecanismos del dolor en los pacientes ya agrupar a los individuos en consecuencia. Hasta ahora, no hay biomarcadores de los mecanismos del dolor, por esa razón, los clínicos tienen que confiar en los marcadores de sustitución que se cree que son iguales a los mecanismos de generación de dolor. Una estrategia potencial para subgrupos de pacientes es emplear el perfil de los síntomas sensoriales y las características del dolor, el denominado fenotipo sensorial, como una medida de las anomalías en las vías de procesamiento del dolor. Tanto las pruebas científicas de animales como las humanas apoyan este enfoque en NP. Está claro ahora que la lesión nerviosa provoca multitud de cambios moleculares y funcionales en cada constituyente de la vía nociceptiva. Como resultado, las fibras nociceptivas desarrollan sensibilidad irregular y actividad espontánea. Presumiblemente, estos cambios en el sistema nervioso del paciente conducen a la percepción de sensaciones de dolor de disparo, dolor espontáneo, además de hiperalgesia térmica. Después de anomalías somatosensoriales adicionales, periféricas y centrales, puede ocurrir el desarrollo de un perfil somatosensorial único y distintivo para cada individuo.

Con el fin de validar la idea de tratamiento basado en mecanismos, tiene que demostrarse que los grupos de personas con un fenotipo sensorial específico realmente responden de manera diferente a un tratamiento determinado. Hasta la fecha, los ensayos clínicos diseñados prospectivamente que se han llevado a cabo utilizando el método de fenotipificación sensorial como un criterio de estratificación y el análisis de respuesta retrospectiva utilizando el fenotipado sensorial de los pacientes con NP al inicio del ensayo son muy prometedores (Herrmann et al., 2006, Baron et al. 2012, Yarnitsky y otros, 2012, Martínez y otros, 2013, Cruz Almeida y Fillingim 2014, Höper et al., 2014, Mainka et al., 2016). Sin embargo, esta técnica debe ser implementada en los siguientes diseños de estudios para eventualmente validar el concepto de tratamiento basado en mecanismos. Además, se deben desarrollar técnicas de evaluación más sencillas para identificar con fiabilidad los subgrupos de pacientes con NP en la práctica clínica general.

Tratamiento farmacológico para el dolor neuropático.

Durante la última década, se han sugerido varias recomendaciones para la farmacoterapia de NP o ciertas condiciones NP, especialmente neuralgia post-herpética y neuropatías diabéticas dolorosas (Finnerup et al., 2015, Dworkin et al., 2007, Attal et al. 2010, Bril et al., 2011). Al mismo tiempo, se han desarrollado nuevas terapias farmacológicas y se han completado ensayos clínicos de alto nivel. Además, se ha reducido el riesgo de sesgo en los datos de recopilación, análisis y notificación debido al análisis del

sesgo de publicación, junto con la identificación más fácil en línea de los estudios farmacológicos no revelados o no publicados. Se ha demostrado que los diferentes tipos de medicamentos con efectos analgésicos funcionan mejor que el placebo en ensayos clínicos incluyendo pacientes con diversas condiciones de NP que cubren opiáceos, anestésicos locales, antagonistas de receptores NMDA, cannabinoides, antidepresivos, anticonvulsivos, toxina botulínica, capsaicina tópica y varios otros (Finnerup et al., 2015).

Muchos de estos fármacos se desarrollaron inicialmente para otras indicaciones, como la epilepsia o la depresión, y posteriormente se evaluaron en NP. El metanálisis y las revisiones sistemáticas de los ensayos de NP y el desarrollo de guías de manejo por varias asociaciones y sociedades han llevado a las recomendaciones actuales para la gabapentina, la pregabalina, los antidepresivos tricíclicos y los antidepresivos de la recaptación de serotonina-norepinefrina (SNRI) como terapias de primera línea; Recomendaciones débiles para parches de lidocaína, parches de capsaicina de alta concentración, opioides, toxina botulínica A y combinaciones de agentes de primera línea seleccionados; Y recomendaciones débiles contra el uso de cannabinoides y valproato (Finnerup et al., 2015). Parece claro entonces que la matriz de medicamentos, y otras intervenciones de tratamiento, con eficacia demostrada en NP se está expandiendo. Por lo tanto, la investigación futura no sólo debe definir el mejor uso de los medicamentos existentes solo y en asociación, sino que también debe determinar los medicamentos que aumentan la magnitud del alivio del dolor o la probabilidad de una respuesta beneficiosa.

Terapia Invasiva para el Dolor Neuropático.

Los pacientes con NP con frecuencia no responden lo suficiente a la medicación usada sola o en combinación con tratamientos no farmacológicos y su dolor es usualmente como resultado refractario (Smith et al., 2012). En lugar de continuar la rotación farmacológica interminable que no ofrece la reducción del dolor deseada o los efectos secundarios indeseables de origen, los tratamientos intervencionistas pueden ser contemplados. Las técnicas de manejo del dolor intervencionistas abarcan el bloqueo neural, la estimulación de la médula espinal, la medicación intratecal y las intervenciones neuroquirúrgicas (Día 2008, Dworkin et al., 2013, Pereira y Aziz 2014). Una revisión sistemática actualizada analizó la eficacia de varias técnicas invasivas en varias condiciones de dolor neuropático, tales como neuralgia post-herpética, dolor neuropático de la lesión medular y dolor de postrada central, neuropatías diabéticas dolorosas y otras neuropatías periféricas, radiculopatía y síndrome de cirugía de espalda fallida, Neuralgia del trigémino y neuropatía trigeminal y CRPS (Dworkin et al., 2013).

Este estudio concluyó que las pruebas para la efectividad de las técnicas intervencionistas en NP son limitadas. Se demostró que casi la mitad de los pacientes obtener alivio parcial duradera del dolor, incluso un número menor de aquellos cuyo alivio fue completa. Sin embargo, es importante resaltar que la evaluación cuidadosa de las opciones de dolor intervencionista y quirúrgico está plagada de numerosos riesgos de sesgo asociados al obstáculo ético y práctico al cegamiento del tratamiento y al empleo de una falsificación óptima u otras intervenciones de control; Estudiar los abandonos de los pacientes atribuibles a la intratable o severidad ya los individuos tratados; Y la logística y los costos de seguimiento y duración del estudio. Además, debe recordarse que el déficit relativo de evidencia de eficacia no obligatoria indica evidencia de falta de eficacia y por lo tanto, el manejo racional de la intervención de los estados NP debe

ser contemplado como un componente integral de un enfoque más amplio que abarca farmacológico y no farmacológico y no intervencionista.

El enfoque multimodal y las terapias no farmacológicas.

Ningún tratamiento farmacológico o de intervención suprime totalmente los síntomas de NP. Un enfoque de gestión completo implica preferentemente el uso de estrategias de tratamiento adicionales en un esfuerzo por mejorar la situación. Los objetivos de cualquier terapia de dolor incluyen no sólo mejorar los síntomas, sino también recuperar la función física, reducir la angustia psicológica, y mejorar la calidad de vida en general. Es necesario que los clínicos luego aclaren estos objetivos a los pacientes, establezcan las expectativas apropiadas para el alivio del dolor, e involucren a los individuos como actores activos en su manejo. Además, el médico debe comprender los beneficios de la gestión multidisciplinaria, facilitando la participación activa en este enfoque multifacético. Se ha demostrado que las personas tratadas en centros de dolor multidisciplinarios redujeron la intensidad del dolor más rápidamente, redujeron el uso de opioides y mejoraron la calidad de vida relacionada con la salud en comparación con los pacientes tratados por médicos generales incluso cuando se adhieren a un plan de control del dolor delineado por un especialista en dolor (Becker et al., 2000). Neurología, psicología, fisioterapia, terapia ocupacional y apoyo social contribuyen al resultado final de los pacientes con ChP.

Asistir al componente psicológico del NP crónico es crucial para el éxito. Los clínicos pueden apoyar a los pacientes muy fácilmente, simplemente centrándose en la funcionalidad y el compromiso normal en las actividades. El catastrófico del dolor, es un predictor bien conocido de la mala respuesta a la farmacoterapia y una mayor probabilidad de interrupción del tratamiento; Catastrófica también predice duración más extensa del dolor, mayor grado de discapacidad y peor calidad de vida (Smeets et al., 2006, Toth et al., 2014). La terapia cognitivo-conductual aborda directamente este comportamiento desadaptativo, ayudando a los pacientes a refinar las emociones y los pensamientos a través de la educación y el entrenamiento en habilidades de afrontamiento y / o confrontación consciente de comportamientos y pensamientos dañinos (Turk 2003, Nijs y otros, 2014). Este tipo de enfoque psicológico ha demostrado proporcionar un beneficio en los pacientes NP no sólo la reducción de la intensidad del dolor, sino también proporcionar una mejora duradera en la funcionalidad, la afrontamiento, el dolor relacionado con el comportamiento, la ansiedad y la participación de los pacientes que sufren de dolor neuropático crónica Et al., 2003, Heutink et al., 2014). Además, la intervención psicológica está siendo mejorada por la presencia de un apoyo social adecuado. Por esa razón, las terapias de grupo de apoyo se han adoptado en el manejo del dolor, facilitando el compromiso del paciente en la estrategia de manejo, permitiendo a los pacientes compartir técnicas de afrontamiento y proporcionar un ambiente de apoyo para el refuerzo positivo. Otros programas de bienestar psicoeducativo que aumentan la conciencia de los factores sociales, intelectuales, emocionales y espirituales también son eficaces para mejorar la calidad de vida general y el bienestar de los individuos con dolor (Subramaniam et al., 1999, McGuire y otros, 2015).

NP, como se mencionó anteriormente, por lo general conduce a reducir la actividad física y funcionalidad. El tratamiento eficaz debe dirigirse tanto a la mejora de los síntomas como a la restauración y movilización funcional. Un enfoque multidisciplinario de cualquier condición NP es importante; Por ejemplo, en la neuropatía diabética recuperar la movilidad y la funcionalidad es crucial para evitar las

úlceras, contracturas y pérdida de sensibilidad. Además, el entrenamiento físico en estos pacientes mejoró las limitaciones funcionales percibidas, la fuerza muscular y la regulación de la glucosa en la sangre (Otterman et al., 2011). Otras modalidades físicas como la estimulación nerviosa eléctrica transcutánea o el uso de técnicas osteopáticas pueden ser aplicadas para mejorar la NP crónica (Kumar y Marshall 1997, Kuchera 2007, Arienti et al., 2011). La fisioterapia para la restauración funcional también es comúnmente aceptada como uno de los pilares en el manejo de CRPS (Zernikow et al., 2012).

Dolor neuropático en los niños

El CHP afecta aproximadamente al 6% de los niños y adolescentes (van Dijk et al., 2006). El porcentaje de estos niños que desarrollan NP es desconocido. Algunas personas sugieren que la prevalencia de ChP con características neuropáticas en la edad adulta es similar a la encontrada en niños (5% -10%) (Torrance et al., 2006, van Hecke et al., 2014). Sin embargo, la evidencia actual indica que a pesar del hecho de que NP se ve en una parte notable de referencias a clínicas pediátricas de ChP, la prevalencia es mucho más baja (Martin et al., 2010, Borsook 2012) y las condiciones con las que está vinculada varían de aquellas Generalmente descrito en la edad adulta.

Las causas aceptadas de NP durante la niñez abarcan desde situaciones en las que se han producido daños en los nervios, por ejemplo, dolor post-traumático, de miembro fantasma, post-quimioterapia y en algunos estados crónicos o infecciones como VIH / SIDA a condiciones genéticas que afectan la función nerviosa sensorial, Y la eritromelalgia (Ramaswami 2008, Fischer y Waxman 2010, Walco et al., 2010). CRPS es una condición idiopática importante que ocurre en niños y adultos que generalmente se cree que se caracteriza por NP.

Así se ha detectado evidencia de NP en la infancia, siendo neuropatía y disfunción del nervio sensorial características principales de la mayoría de las afecciones neuropáticas. Sin embargo, es evidente a partir de nuestro conocimiento actual de la prevalencia y el pronóstico que hay diferencias significativas en la respuesta sensorial a la lesión nerviosa y los daños del SNC que parecen estar fuertemente relacionados con la edad de desarrollo. A modo de ilustración, varios ensayos clínicos en animales han estudiado los efectos de diversos tipos de lesión nerviosa en diferentes condiciones de NP y edades de desarrollo, mostrando cómo los mecanismos que operan en NP infantil pueden variar de aquellos en edad avanzada. Los modelos de laboratorio de lesión traumática del nervio periférico han confirmado una susceptibilidad disminuida a NP si el daño aparece en una edad más joven; Además, estos hallazgos se han utilizado para mejorar la comprensión de los cambios relacionados con la edad en la fisiopatología de NP (Howard et al., 2005, Walker et al., 2009). Del mismo modo, la literatura actual sugiere que los síntomas de NP en la infancia son generalmente menos frecuentes y su gravedad se relaciona con la causa subyacente, la edad de inicio o la duración de la enfermedad (Vogel y otros 2002, Atherton et al., 2008).

Las recomendaciones actuales para la evaluación y diagnóstico de NP están configuradas para adultos, sin embargo, generalmente se extrapolan a niños o adolescentes. Como no hay pruebas definidas de biomarcadores para NP el diagnóstico se hace sobre la base de indicadores clínicos que es un tema bien conocido de la evaluación del dolor en niños pequeños. La historia clínica sigue siendo en la infancia la pieza central del diagnóstico; Sin embargo, los niños pueden emplear descriptores cualitativos que se consideran indicativos de NP, tales como disparos, radiación, ardor, pinchazos, hormigueo, electrochoque, apuñalamiento, pinchazos y pinzamiento (Krane y Heller, 1995, Walco Et al., 2010).

Obviamente, muchos niños pueden no ser capaces de describir su dolor usando estos términos, pero en cualquier caso el historial del dolor debe contener la evaluación de la intensidad, la calidad, los aspectos temporales del dolor y la respuesta al tratamiento (Haanpää et al. Preferiblemente, todas estas características -pero lo más importante, la intensidad del dolor- deben ser evaluadas usando una escala validada; Desafortunadamente, las escalas observacionales se han diseñado principalmente para aplicarse en los ajustes de dolor agudo en adultos y pueden no ser confiables.

Al igual que en los adultos, la evaluación física debe apuntar a identificar, confirmar y localizar el daño del sistema somatosensorial e informar cualquier signo neurológico asociado. Las anomalías sensoriales son más complicadas de adquirir en los niños por muchas razones, como la comunicación o la falta de datos previos. Por ejemplo, métodos confiables como la QST evalúan los patrones de cambio en asociación con NP en adultos, además de que es muy importante establecer una buena comunicación entre el paciente y el investigador, ambos rasgos que usualmente disminuyen al evaluar a un niño. La microneurografía, la imagen cerebral funcional, la electroneuromiografía y la biopsia cutánea pueden estar indicadas, aunque nuevamente su uso se limita principalmente a la investigación (Lebel et al., 2008). Además, la evaluación de la discapacidad relacionada con el dolor, el sueño, la calidad de vida, el funcionamiento del papel y el estado de ánimo deben ser evaluaciones habituales para los niños que sufren de NP.

Eltratamineto de la NP en las edades jóvenes es aún más difícil y refractario que en los adultos. Como se mencionó anteriormente, el NP crónico en raras ocasiones responde aceptablemente a una sola medicación analgésica o estrategia de manejo del dolor. La mayoría de los estados de dolor están altamente modulados por la actividad en el SNC-cerebro y médula espinal, que a su vez están afectados por factores cognitivos y emocionales y por lo tanto un abordaje multimodal basado en un modelo de dolor biopsicosocial siempre puede ser contratado. Por lo tanto, es probable que los avances en el manejo de PN sean consecuencia de una mejor comprensión de los mecanismos heterogéneos implicados en diferentes etapas de desarrollo, mejoras en la precisión del diagnóstico clínico y un enfoque documentado mucho más sistemático y cauteloso de la terapia. El manejo correcto debe abarcar ciertos fármacos analgésicos idealmente elegidos sobre la base de los mecanismos de dolor que se cree que están implicados y una gama de estrategias de manejo del dolor adecuadas para la evaluación clínica del dolor y cualquier deterioro funcional relacionado. La farmacoterapia en NP suele ser empírica y decepcionante, ya que el mecanismo subyacente particular rara vez se entiende completamente. Aun así, es razonable un enfoque general basado en una evaluación de los beneficios previsibles balanceada.

Condiciones especiales. CRPS

El síndrome de dolor regional complejo (CRPS) es un término acuñado por la Asociación Internacional para el Estudio del Dolor (IASP) para designar estados caracterizados por dolor espontáneo o inducido por estímulo que es desproporcionado con el evento incitante y asociado con una amplia variedad de Motora y autonómica en combinaciones muy variables (Harden et al., 2007). La evidencia apunta a que la etiología del CRPS es un trastorno multifactorial que está asociado con una respuesta aberrante del huésped a la lesión tisular. La variación en la susceptibilidad a la regulación perturbada de cualquiera de las vías biológicas subyacentes probablemente explica la heterogeneidad clínica del CRPS (Marinus et al., 2011). Existen muchos aspectos fisiopatológicos etiológicos que se han acusado en el desarrollo de la CRPS, sin embargo se han identificado tres vías fisiopatológicas principales: mecanismos inflamatorios

aberrantes, disfunción vasomotora y neuroplasticidad maladaptativa. Además, parece claro que las diferencias interindividuales en la medida en que estos mecanismos están afectados explican la heterogeneidad clínica del trastorno (Bruehl 2010).

Este síndrome está lleno de incertidumbre y comúnmente inexacta. No existen pautas totalmente aceptadas que puedan utilizarse para el diagnóstico y llenarán las definiciones de la medicina basada en la evidencia. De hecho, hay casi tantos criterios de diagnóstico como hay denominaciones a esta condición. El término paraguas CRPS puede separarse actualmente en tipos I y II. CRPS I pretende abarcar la distrofia simpática anteriormente conocida y trastornos similares sin una lesión nerviosa; Mientras que CRPS II se produce después de un daño a un nervio periférico (Harden et al., 2007, Borchers y Gershwin 2014). Hasta la fecha, la cantidad de ensayos que han incluido controles apropiados y tienen un número suficiente de participantes para permitir el análisis estadístico con cálculos de potencia aceptables es menor.

Esto tiene como resultado un diagnóstico excesivo y, a menudo, el uso de una farmacoterapia excesiva e incluso intervenciones quirúrgicas innecesarias (Maihöfner et al., 2010).

En la última década también se ha convertido en una entidad bien establecida en niños y adolescentes, aunque con problemas similares a los adultos (Berde y Lebel 2005). CRPS sigue siendo probablemente diagnosticado y no distinguir CRPS lleva a retraso en el manejo, las pruebas diagnósticas innecesarias y el tratamiento inadecuado, lo que puede empeorar la situación y agravar el sufrimiento. El diagnóstico precoz, la derivación y el tratamiento adecuados son esenciales para disminuir el dolor y mejorar la función de los niños con SDRC (Stanton-Hicks 2010, Logan et al., 2013).

Por estas razones hemos dividido el estudio en tres partes.

La primera de las tres partes de las que se compone este trabajo tiene como objetivo el reconocimiento de cambios o hallazgos no descritos en el sistema somatosensorial en un modelo experimental de DN.

Primer Estudio. La alteración de la distribución y composición de los canales de potasio en los axones mielinizados suprime la hiperexcitabilidad después de la lesión.

El DN tras un daño nervioso periférico esta asociado con la hiperexcitabilidad de los axones sensoriales mielinizados afectos, la cual comienza a normalizarse con el tiempo. Este trabajo investiga la composición y distribución de los canales de potasio voltaje dependientes de la familia Shaker (Kv1) en el complejo nodal de los axones mielinizados tras la lesión nerviosa. En el neuroma que se forma tras el daño, la expresión de Kv1.1 y Kv1.2 estaba considerablemente reducida. Por el contrario Kv1.4 y Kv1.6, que eran casi imperceptibles en el estado naive, mostraron un importante aumento en su expresión tras el daño. En la raíz dorsal se pudo demostrar una redistribución de los canales Kv1 hacia el paranodo. El bloqueo de los canales Kv1 con a-DTX tras la inducción de la lesión reinstauró la hipersensibilidad de las fibras A y aumentó la sensibilidad mecánica. Los cambios observados en la composición molecular y distribución axonal de los canales Kv1, representan por tanto un mecanismo protector que disminuye la hiperexcitabilidad de los axones sensoriales mielinizados tras un daño nervioso.

Actualmente, tanto la evidencia animal como la humana coinciden en que para el desarrollo del DN una lesión de las vías aferentes somatosensoriales es necesaria. Además, numerosos estudios demuestran que son varios los mecanismos que pueden inducir el desarrollo del DN. Esto no solo pone de manifiesto

la complejidad del DN, sino que también destaca la importancia clínica de identificación de los mecanismos subyacentes del dolor en cada uno los individuos que lo sufren. Ya que diferentes planes de tratamiento son necesarios para el manejo de los diferentes mecanismos, un abordaje terapéutico basado mecanismos podrá guiar a los profesionales de la salud hacia mejores resultados.

Este trabajo muestra que dentro de un neuroma, los niveles de expresión de Kv1.1 y 1,2 están marcadamente reducido, pero con el tiempo Kv1.4 y 1,6 expresión aumenta dentro de yuxtaparanodos y paranodos. En sitios alejados de la lesión, también hay una redistribución gradual de canales Kv1 al paranodo. Los experimentos electrofisiológicos y de comportamiento sugieren que los cambios en la expresión de la subunidad y la redistribución de los canales Kv1 actúan como un "freno" sobre el estado hiperexcitable que surge en los axones mielinizados después de la lesión traumática del nervio.

Hemos encontrado cambios importantes en la composición y distribución de la subunidad de los canales Kv1 dentro del axolemma de los axones mielinizados tras la lesión traumática del nervio. En contraste con el soma en el que se reduce la expresión de los canales Kv1, esta mayor disponibilidad de canales Kv1 dentro de los paránodos y la composición de la subunidad alterada parece cumplir un papel adaptativo en la supresión de la excitabilidad excesiva en las aferencias mielinizadas.

Segundo estudio. Perfiles de síntomas en el cuestionario de dolorDETECT en pacientes con dolor neuropático periférico estratificados según pérdida sensorial en pruebas sensoriales cuantitativas

El dolorDETECT cuestionario (PDQ) se desarrolló y validado para apoyar la identificación de componentes de dolor neuropático en pacientes que sufren de dolor crónico de origen diferente (Freynhagen et al., 2006). Se ha demostrado previamente que los descriptores de dolor del PDQ se correlacionan con los ítems de QST que prueban umbrales de dolor relacionados en pacientes que sufren de radiculopatía, pero no en pacientes que padecen fibromialgia (Tampin et al., 2013). Se realizó este análisis exploratorio en un estudio prospectivo de muestra mayor debido a que el PDQ fue desarrollado antes de que se estableciera una definición clara de NP (Treede et al., 2008). Por lo tanto, nunca fue parte del proceso de validación del PDQ para investigar si diferentes tipos de pérdida sensorial (como se describió anteriormente) presentan con diferentes perfiles PDQ o si los elementos PDQ individuales son sensibles a los tipos de pérdida sensorial en un examen clínico. Esto sería útil para validar el PDQ no sólo como una herramienta de cribado para la NP misma, sino también para diferentes subtipos sensoriales de NP. El objetivo del estudio fue analizar si el puntaje total del PDQ o sus ítems reflejan los fenotipos de pérdida sensorial en NP según lo determinado por la QST.

Dentro del consorcio europeo Europain y Neuropain, tanto los datos QST como los PDQ de los pacientes con dolor neuropático evaluados por unidades de investigación del dolor en toda Europa se recogieron en una base de datos central, permitiendo un análisis de la correlación entre la QST obtenida somato-Perfiles sensoriales de pérdida de función térmica y / o mecánica y perfiles PDQ.

Nuestros resultados demuestran que la puntuación PDQ no es lo suficientemente sensible como para distinguir los diferentes tipos de pérdida sensorial en pacientes con NP. Si bien nuestros resultados indican que existen diferencias en las respuestas a cuatro ítems entre grupos de pacientes con uno u otro tipo de pérdida de sensibilidad detectada por la QST, no proporcionan información clara sobre la base de un paciente individual, ya que las diferencias de medias son comparables Pequeño (alrededor de un

punto en una escala de cero a cinco) y muestran una gran superposición. PDQ es incapaz de separar claramente entre los pacientes con diversos tipos de pérdida de sensación, porque sus preguntas para la pérdida de función se limitan a entumecimiento (y están completamente ausentes en otros cuestionarios de dolor), mientras que la pérdida de función puede ser detectada para todas las cualidades sensoriales en QST. Un análisis más complejo, que identifica elementos que diferencian los tipos de pérdida sensorial en una nueva batería de preguntas, sería un enfoque interesante para un estudio futuro. Algunos dominios pueden dar información más detallada si se dividen en dos elementos, por ejemplo, el tema de dolor frío o calor, uno para el dolor evocado por el frío y otro por el dolor evocado por el calor. Artículos separados para las diferentes formas de hiperalgesia o alodinia también podría ser de valor. Además, las preguntas sobre los trastornos autonómicos, que comúnmente aparecen en pacientes con neuropatía de fibra pequeña (Lacomis 2002), sería útil. Los resultados de nuestro estudio concluyen que los pacientes con perdida significativa de sensación térmica reportaban mas frecuentemente dolor evocado por tacto fino, y los pacientes con perdida significativa de sensibilidad mecánica reportaban mas frecuentemente adormecimiento, así como una menos frecuente sensación de quemazón y dolor evocado por tacto fino. A pesar de que el PDQ no fue diseñado para evaluar la perdida de sensibilidad, sus ítems individuales pueden reflejar la perdida de sensibilidad térmica y/o mecánica, pero debido a la importante variabilidad existente, el PDQ no nos permite la asignación de pacientes en perfiles sensoriales. La continuación de este paso en el desarrollo de una herramienta de cribado conveniente podría ayudar a identificar diferentes fenotipos involucrados en NP.

Tercer estudio. Síndrome de dolor regional complejo en niños.

I.Un enfoque multidisciplinario y técnicas invasivas para la gestión de los no respondedores

Síndrome de dolor regional complejo (CRPS) es un término refinado por la Asociación Internacional para el Estudio del Dolor (IASP) para describir trastornos caracterizados por dolor espontáneo o inducido por estímulo que es desproporcionado con el evento incitante (Swart et al., 2009, Bruehl 2010 , Borchers y Gershwin 2014). CRPS ha sido ampliamente estudiado en adultos, pero los estudios en niños son escasos. El presente artículo informa sobre el curso y el manejo de 10 niños diagnosticados con SDRC que no respondieron con éxito a las terapias conservadoras del manejo del dolor que se presentan en nuestra Clínica del Dolor.

Este estudio ofrece un enfoque de manejo multidisciplinario para el tratamiento de la SDRC en niños para quienes el tratamiento estándar no tuvo éxito. Debido a la gravedad y rápida progresión de los síntomas en la CRPS, consideramos que el diagnóstico temprano de la enfermedad junto con un tratamiento multidisciplinario integral e individualizado ofrece a los niños con CRPS la mejor oportunidad para una recuperación completa. Dentro de este plan de manejo, deben incluirse nuevos fármacos, como el parche de capsaicina al 8%, además de técnicas invasivas para pacientes que de otro modo no responden a terapias no invasivas. Por lo tanto, un enfoque más agresivo debe ser intentado. Llegamos a la conclusión de que se necesita más investigación sobre el SDRC en niños y se requieren nuevas pautas de tratamiento para aquellos niños que no responden a las modalidades de manejo establecidas.

PUBLICATIONS

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RESEARCH ARTICLE

Altered potassium channel distribution and composition in myelinated axons suppresses hyperexcitability following injury

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Abstract Neuropathic pain following peripheral nerve injury is associated with hyperexcitability in damaged myelinated sensory axons, which begins to normalise over time. We investigated the composition and distribution of shaker-type-potassium channels (Kv1 channels) within the nodal complex of myelinated axons following injury. At the neuroma that forms after damage, expression of Kv1.1 and 1.2 (normally localised to the juxtaparanode) was markedly decreased. In contrast Kv1.4 and 1.6, which were hardly detectable in the naïve state, showed increased expression within juxtaparanodes and paranodes following injury, both in rats and humans. Within the dorsal root (a site remote from injury) we noted a redistribution of Kv1-channels towards the paranode. Blockade of Kv1 channels with α-DTX after injury reinstated hyperexcitability of A-fibre axons and enhanced mechanosensitivity. Changes in the molecular composition and distribution of axonal Kv1 channels, therefore represents a protective mechanism to suppress the hyperexcitability of myelinated sensory axons that follows nerve injury.
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eLife digest Around 20% of the world's population experiences long-lasting "chronic" pain, which often results in poor sleep, depression and anxiety. One of the most disabling forms of chronic pain is called neuropathic pain, which results from injuries to sensory nerves. Pain or discomfort is felt in response to touches that are not normally painful. Neuropathic pain is difficult to treat as we do not fully understand the molecular mechanisms that cause it.

Stimulating a nerve causes it to produce action potentials. At a molecular level, these action potentials are generated by ions moving into and out of the neuron through proteins called ion channels. The movement of sodium ions into a neuron triggers an action potential, and the movement of potassium ions out of the neuron returns it to a resting state.

After a sensory nerve is cut or otherwise damaged it becomes hyperactive and produces spontaneous electrical activity that the brain interprets as pain signals. However, it is not fully understood how cutting a nerve affects the ion channels in a way that generates this hyperactivity.

Different types of ion channel are found in different regions of the nerve cell; for example, type 1 potassium channels are normally found in a region called the juxtaparanode at the axon of the neuron. Calvo et al. have now tracked what happens to type 1 potassium channels after nerve injury in rats. Soon after nerve damage occurred, nearly all of these ion channels disappeared from the juxtaparanode. At the same time, electrical activity in the cut nerve increased, and the recovering animals responded in ways that suggested they were hypersensitive to the nerve being touched.

Three weeks after the injury, most rats lost their hypersensitivity and the electrical activity in the cut nerve returned to near-normal levels. Calvo et al. found that the recovering nerves contained new subtypes of type 1 potassium channels. These potassium channels did not just appear in the juxtaparanode: they also invaded the 'fence' region that normally separates potassium channels from sodium channels. The same was observed to happen in the nerves of patients that suffer from neuropathic pain due to a nerve injury.

At this late time point after nerve injury, blocking the activity of potassium channels produced the same abnormal increase in the nerve's electrical activity as seen immediately after the nerve had been cut. The rats' hypersensitivity to touch also returned. This suggests that the appearance of the new potassium channel subtypes might be a protective mechanism that reduces the activity of a damaged nerve to decrease pain.

These findings suggest new ways of treating neuropathic pain. Further studies are now needed to investigate whether drugs that can activate the new potassium channel subtypes could stop pain from an injured nerve becoming a long-term problem.

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Introduction

Following traumatic nerve injury spontaneous activity develops initially in myelinated and subsequently in unmyelinated sensory axons (***Wall and Gutnick, 1974***; ***Kajander and Bennett, 1992***; ***Boucher et al., 2000***; ***Michaelis et al., 2000***; ***Wu et al., 2001***; ***Liu et al., 2000a***). The onset of this spontaneous activity is associated with the emergence of pain-related sensory changes in animal models and is critical for the maintenance of peripheral neuropathic pain (***Haroutounian et al., 2014***) in patients where selective blockade suggests the involvement of myelinated axons (***Campbell et al., 1988***). Ectopic activity is particularly prominent in myelinated afferents and peaks within the first few days post injury and then declines over subsequent weeks (***Kajander and Bennett, 1992***; ***Liu et al., 2000a***; ***2000b***; ***Han et al., 2000***). Such ectopic activity arises both at the neuroma site and also at the level of the dorsal root ganglion (DRG) (***Han et al., 2000***; ***Liu et al., 2000b***; ***Amir et al., 1999***; ***2005***; ***Wall and Devor, 1983***).

Altered expression, function and trafficking of voltage-gated ion channels are key determinants of these excitability changes. *Shaker* type voltage-gated potassium channels (Kv1 channels) are important determinants of neuronal excitability. They are formed by heteromultimers of α and β subunits (***MacKinnon, 1991***). The characteristics of the outward currents they carry depend on subunit composition. Sensory neurons are known to express Kv1 channels and functionally these channels have been shown to limit excitability of sensory neurons: For instance Kv1.2 suppresses excitability

at the level of the sensory neuron cell body (**Gold et al., 1996**; **Rasband et al., 2001**; **Zhao et al., 2013**; **Everill et al., 1998**) and Kv1.1 acts as a 'brake' on mechanosensitivity at the terminals of C-mechano-nociceptor and Aβ-mechanoreceptors (**Hao et al., 2013**). Kv1 channels also act as excitability brakes for cold thermal sensitivity in intact and damaged axons of primary sensory neurons (many of such fibres are also mechano-sensitive) (**Roza et al., 2006**; **Madrid et al., 2009**). Kv1 channels are known to be expressed in the juxtaparanodal region of myelinated sensory axons. An unexplored issue, however, is whether the distribution of these channels changes under pathological neuropathic states.

Saltatory conduction in myelinated fibres depends on the molecular organization of channel domains within the axon (**Chang and Rasband, 2013**): voltage-gated sodium channels (Nav) are clustered at the node of Ranvier. Nodes are flanked by the paranode, which is an important point of attachment between the axon and the terminal loops of the Schwann cell. Just inside the innermost axoglial junction of the paranode is the juxtaparanode a domain enriched in Kv1 channels Kv1.1 and 1.2. The localisation of Kv1.1 and 1.2 to the juxtaparanode is dependent on the formation of a molecular scaffold, which includes the adhesion molecules caspr2 and TAG-1 (**Poliak et al., 2003**). In the naïve state in adulthood, the juxtaparanodal Kv1 channels are thought not to have a major influence on axon conduction properties of peripheral myelinated axons (**Poliak et al., 2003**; **Chiu and Ritchie, 1980**; **Sherratt et al., 1980**; **Rasband et al., 1998**), probably because they are electrically insulated from the node of Ranvier under the myelin sheath. However during development (**Vabnick et al., 1999**) and following primary demyelination (**Rasband et al., 1998**) (during which myelin is removed but the axon remains intact), Kv1.1 and 1.2 become more widely distributed to include the paranode and even the node (**Arroyo et al., 2004**), and can act to suppress excitability. Although Kv1.1 and 1.2 expression within the soma is known to be down-regulated following axon transection, and this leads to hyperexcitability at the soma (**Rasband et al., 1998**; **Ishikawa et al., 1999**; **Park et al., 2003**), the distribution of these channels at the nodal complex and damaged nerve terminal (in the neuroma that forms) has not been examined. Furthermore, little is known regarding the distribution of other members of the shaker type Kv1 channels family such as Kv1.4 and 1.6 following nerve injury.

Here we show that within a neuroma, expression levels of Kv1.1 and 1.2 are markedly reduced but over time Kv1.4 and 1.6 expression increases within juxtaparanodes and paranodes. At sites remote from injury, there is also a gradual redistribution of Kv1 channels to the paranode. Electrophysiological and behavioural experiments suggest that changes in subunit expression and redistribution of Kv1 channels act a 'brake' on the hyperexcitable state that arises in myelinated axons following traumatic nerve injury.

Results

Expression of Kv1 channels subunits switches at nodal regions after nerve injury

To investigate the role of Kv1 channels in hypersensitivity after nerve injury we used a model of complete sciatic nerve transection followed by positioning of the proximal stump superficially under the skin of the leg [modified version of **Dorsi et al. (2008)**]. This model enables us to study both the expression of Kv1 channels within the neuroma and undertake behavioural analysis using specific blockers of Kv1 channels.

To study how the localisation of Kv1 channels changes within the nodal complex, we used a pan-Nav channel antibody to label the node of Ranvier, a Caspr antibody to label the paranode and Kv1.2 and Caspr2 antibodies to label the juxtaparanode. In the naïve axon, we observed that over 90% (mean 91 ± SEM 2.9%, n = 4 animals) of the nodes presented a characteristic morphology with Nav clustering in the centre, surrounded by caspr at both sides and Kv1.2 clustered within the juxtaparanode. Of other members of the Kv1 channels family, Kv1.4 and 1.6 are expressed by DRG cells (**Everill et al., 1998**; **Thakur et al., 2014**; **Chiu et al., 2014**). We found that in the naïve state Kv1.4 was only expressed within a very small proportion of nodes (5.5 ± 4.6%, n = 4 animals) within the juxtaparanode and Kv1.6 was not present within the nodal complex (**Figure 1**).

At the site of the neuroma (day 7 and 21), only half of the nodes demonstrated this typical morphology (day 7 = 47.5 ± 5.1%, day 21 = 46 ± 6.%, n = 4 animals per group, 36–64 nodes per animal);

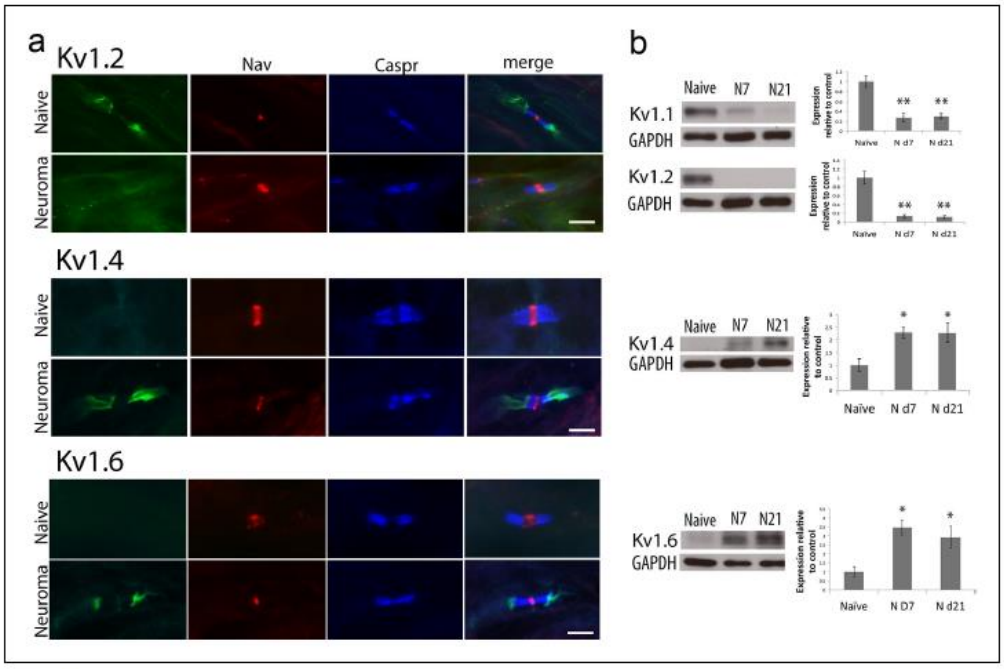

Figure 1. Kv1 channels expression in the naïve nerve and 21 days after sciatic nerve axotomy (note that the samples were collected from the neuroma site). (a) Representative images of longitudinal nerve sections immunostained with Kv1 channels in green (Kv1.2, Kv1.4 and Kv1.6 respectively), a panNav antibody in red (to identify the node), and caspr in blue (to identify the paranode). Kv1.2 is expressed in the juxtaparanode in naïve nerves but it is not present at 21 days after nerve injury. Kv1.4 and kv1.6 are not present in uninjured nerve but are expressed after nerve injury. Note that when Kv1.4 and Kv1.6 are expressed, they are not confined to the juxtaparanode only but invade the paranode. (b) Western blots showing expression of Kv1 channels in the naïve nerve, at 7 and 21 days after axotomy. Kv1.1 and Kv1.2 are expressed in the naïve nerve and down-regulated after axotomy, while Kv1.4 and Kv1.6 have a low/null expression in the naïve nerve and are up-regulated after injury (*p<0.05, **p<0.001, one Way ANOVA, n=6 per group). Scale bars = 5 µm.

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the rest were split (day 7 = 23.3 ± 5.9%, day 21 = 13.5 ± 2.7%), presented as heminodes (caspr at one side only, day 7 = 21.8 ± 4.4%, day 21 = 28.9 ± 5.5%), or were 'naked' (Nav clusters alone, with no caspr, day 7 = 7.2 ± 3%, day 21 = 11.4 ± 3.2%, ***Figure 2***). This is in accordance with previous literature examining the localisation of voltage-gated sodium channels (***Henry et al., 2006***; ***Thakur et al., 2014***). At day 7 after injury, Kv1.2 channels were not located strictly not only in the juxtaparanodal regions but also overlapped with paranodal proteins. To objectively measure this, we quantified the distance between the Nav channels staining and the distal end of the caspr staining, and the distance between the Nav channels staining and the proximal end of Kv1 channels. The difference between these two distances was indicative of the level of overlap between Kv1 channels and paranodal proteins (note that 'naked' nodes were not included in the analysis of the spatial distribution of Kv1 channels because by definition these only consist of Nav clusters without paranodal and juxtaparanodal proteins). In naïve axons the distance between Nav channels staining and the end of caspr was 3.8 ± 0.2 µm, and the distance between Nav channels staining and the start of Kv1.2 staining was 4.2 ± 0.2 µm, resulting in a relatively small, albeit positive, difference between

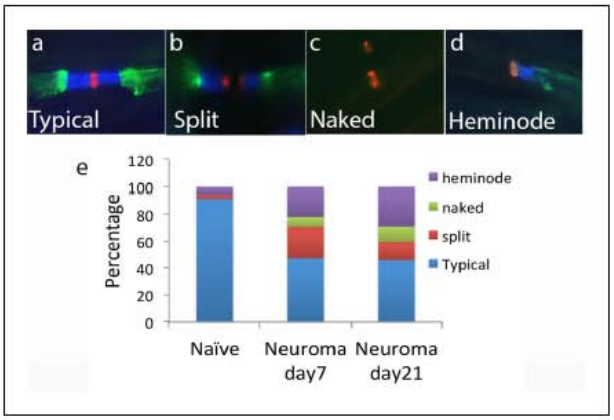

Figure 2. Nav channel expression. Representative sections of longitudinal nerves immunostained with Kv1.2 in green, a panNav antibody in red (to identify the node), and caspr in blue (to identify the paranode) from neuroma day 21. (**a**) A typical pattern of Nav expression localized at the node of Ranvier and flanked by caspr staining is shown. The altered forms of Nav channel accumulations seen in the injured nerve included (**b**) split nodes: These were nodes that had two distinct Nav channels accumulations, separated by a gap in the Nav channels staining within the same fibre and with each Nav channels accumulation flanked on one side with caspr staining, or (**c**) naked nodes: those Nav channel accumulations that lacked an association with caspr (**d**) heminodes: nodes where the caspr staining was located on only one side of a contiguous Nav channel accumulation. (**e**) Quantification of different types of sodium cluster accumulation in the naïve state and after nerve injury is shown.

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these distances (0.5 ± 0.08 µm, n = 4 animals, 25–40 nodes per animal), indicating there was no overlap. At day 7 after injury, the distance between Nav channels staining and the end of caspr was 4 ± 0.1 µm, and the distance between Nav channels staining and the start of Kv1.2 staining was 3.2 ± 0.2 µm, giving a negative value for the difference between both distances (-0.8 ± 0.1 µm, n = 3 animals, 32–35 nodes per animal), which indicates that the Kv1 channels were co-localised with caspr staining and moving closer to the node (***Figure 3***). Note that the distance between Nav channels staining and the end of caspr staining remained unchanged after injury, while the distance between Nav channels staining and the start of Kv1.2 staining was significantly reduced. Contactin-associated protein-like 2 (*Caspr2*) forms a complex with Kv1 channels at the juxtaparanode (***Chiu et al., 2014***). We evaluated if caspr2 moves closer to the node together with Kv1 channels. We measured the distance between the Nav channels staining and the distal end of the caspr staining, and the distance between the Nav channels staining and the proximal end of caspr2. In naïve axons, the distance between Nav channels staining and the end of caspr was 3.8 ± 0.3 µm, and the distance between Nav channels staining and the start of caspr2 staining was 4.3 ± 0.0 µm, resulting in a small difference between these distances (0.49 ± 0.09 µm, n = 4 animals, 25–30 nodes per animal), indicating there was no overlap. At day 7 after injury, the distance between Nav channels staining and the end of caspr was 4.1 ± 0.2 µm, and the distance between Nav channels staining and the start of caspr2 staining was 2.8 ± 0.2 µm, giving a negative value for the difference between both distances (-1.2 ± 0.1 µm, n = 3 animals, 30 nodes per animal), which indicates that the caspr2 co-localised with caspr staining and had moved closer to the node together with Kv1 channels (***Figure 3***).

The redistribution of the channels seen could simply be a reflection of direct injury at the site of axotomy. We therefore, studied a site proximal to the neuroma (1 cm) and compared it to the neuroma site. The effect on Kv12 re-localization remains the same at the site far from the neuroma: The difference between Nav channels staining and the end of caspr distance and Nav channels staining

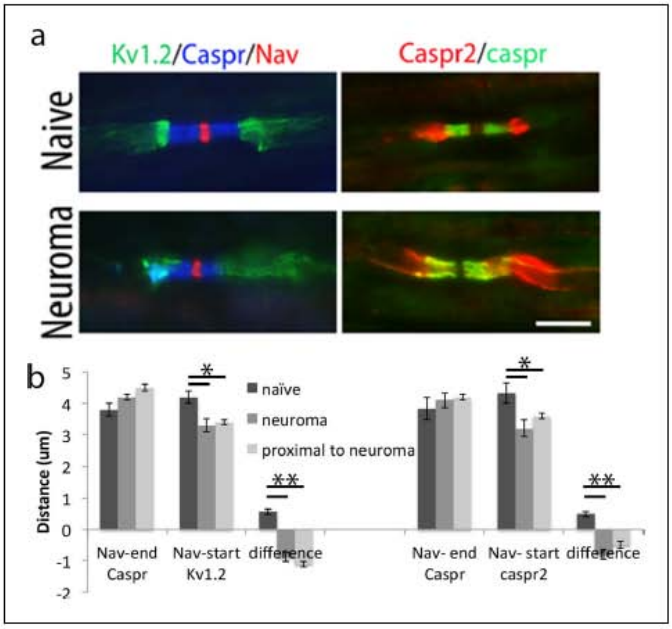

Figure 3. Relocalization ok Kv1.2 and caspr2 at 7 days after neuroma. (a) Representative longitudinal sections of nerves immuno-stained with Kv1.2 in green, a panNav antibody in red (to identify the node) and caspr in blue (to identify the paranode). Kv1.2 is expressed in the juxtaparanode in naïve nerves but it also co-localized with caspr staining at 7 days after injury. Representative longitudinal sections of nerves immuno-stained with caspr2 in green and caspr in red. Caspr2 is confined to the juxtaparanode in naïve nerve but co-localized with caspr at 7 days after injury. (b) We quantified the distance between the sodium channel staining (Nav) and the end of the caspr staining, distance between the sodium channel staining (Nav) and the start of the Kv1.2/1caspr2 staining, and difference between these distances. A negative value represents an overlap of paranodal and juxtaparanodal proteins. Note that he distance between the sodium channel staining (Nav) and the end of the caspr staining remains unchanged after nerve injury, while the distance between the sodium channel staining (Nav) and the start of the Kv1.2/caspr2 staining is significantly shortened after nerve injury, indicating co-localization of Kv1.2 and caspr2 with caspr (n = 5 animals, 20–41 nodes per animal), p<0.001, one way ANOVA Tukey post hoc tests). We analyzed uninjured (naïve) nerve, nerve at the site of the neuroma (day 7), and nerve 1 cm proximal to the neuroma (day 7). The effect on Kv1.2 re-localization remains the same at the site far from the neuroma. The effect on caspr2 re-localization is slightly smaller at the site 1 cm proximal to the neuroma compared with the neuroma site, but it is still significantly different from the naïve **p<0.001, *p<0.05, PRN = paranode, JXP = juxtaparanode. Scale bars = 5 µm.

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and the start of Kv1.2 distance was -1 ± 0.09 µm, at the site close to the neuroma and -1.1 ± 0.09 µm, at the site far from the neuroma (p = 0.6, n = 5 animals, 24–26 nodes per animal). The effect on caspr2 re-localization is slightly smaller at the site 1cm proximal to the neuroma compared with the site close to the neuroma, but it is still significantly different from the naïve (p<0.001): The difference between Nav channels staining and the end of caspr distance and Nav channels staining and the start of caspr2 distance was -1 ± 0.1 µm, at the site close to the neuroma and -0.5 ± 0.1 µm, at the site far from the neuroma (p = 0.06, n = 5 animals, 21–26 nodes per animal, ***Figure 3c***).

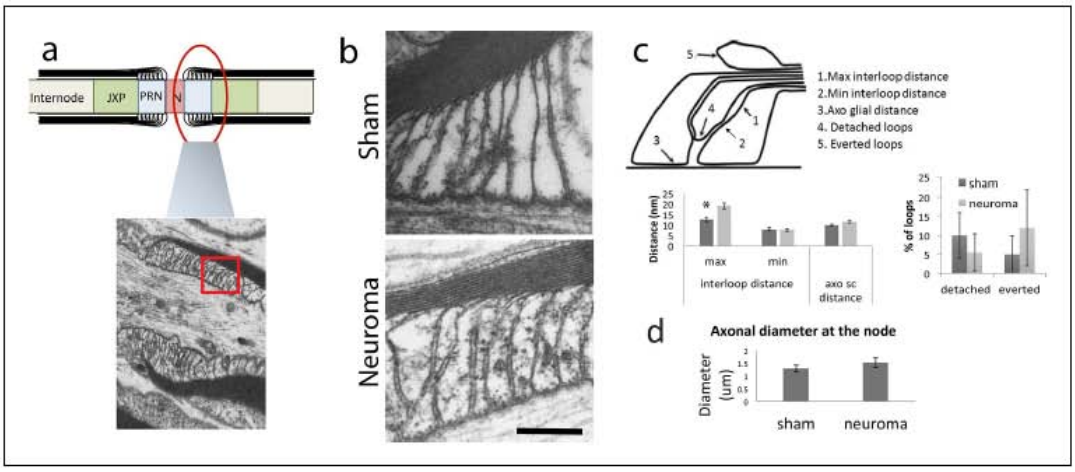

Figure 4. Ultrastructural anatomy of the node of Ranvier within the sciatic nerve following axotomy. We used electron microscopy to look at the ultrastructure anatomy of the node. (**a**) Shows a diagram of the node, paranode and juxtaparanode and a low magnification section of this area in a sham-operated nerve. The red box denotes the area that was used for quantification as seen in **b**. (**b**) High magnification views of the paranodal loops are shown in the sham and 21 days following axotomy (magnification 135 000x) (**c**) We quantified different aspects of the attachment of the Schwann cell paranodal loops to the axon. This is illustrated in right panel which denotes the different parameters measured: The maximal and minimal distance between interloops, the distance between the glia and the axon, the number of detached loops and the number of everted loops. We found a significant increase in the maximal distance between loops in the neuroma compared to sham nerves (one way ANOVA, $p = 0.005$). There were no significant differences in any of the other measurements. (**d**) We quantified the diameter of the axons at the site of the node and found no difference between the uninjured and injured axons. Scare bars: 200 nm.

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This change on juxtaparanode proteins localisation could be due to a disorganisation of the paranodal axo-glial junctions (paranode loops). Therefore, we examined their ultrastructural anatomy using electron-microscopy and measured the distance between the axon and the paranodal loops (axo-glial distance), the number of detached and everted loops and the minimal and maximal distance between loops. We analysed sciatic nerves from sham-operated and neuroma animals and we observed very few detached or everted loops, with no differences between groups. The close apposition between the axon and the paranodal loop was unchanged as the axo-glial distance was not significantly changed (sham = 9.7 ± 0.4 nm, neuroma = 11.4 ± 0.7 nm, n = 4–5 animals per group, 6–9 nodes per animal one way ANOVA $p = 0.08$). We observed a small but significant increase in the maximal distance between loops (sham = 12.5 ± 1 nm, neuroma = 18.9 ± 1.4 nm, one way ANOVA, $p = 0.005$). In summary, these results suggest that although there was no major disruption of the septate axoglial junctions there was a small but significant increased separation between the paranodal loops (***Figure 4***). Note that the axonal diameter at the node did not change (***Figure 4d***).

βII spectrin is a cytoskeletal protein that has recently been shown to be essential for the localization of Kv1 channels to the juxtaparanode and is proposed to form a sub-membranous barrier to lateral diffusion of Kv1 channels into the paranode (***Zhang et al., 2013***). Using IHC, we looked at βII spectrin in the neuroma and found that it is expressed at the paranode and juxtaparanode. We quantified the staining at the paranodal domain and found that the expression of this protein was reduced by more than half compared to naive (immunofluorescence normalised to naïve: 0.4 ± 0.02, $p<0.001$, t-test, n = 50–83 heminodes, a–b). βII spectrin is also expressed in the sub-membranous regions of Schwann cells where it does not appear to change with nerve injury. To further quantify the change of expression of this protein in the neuron, we performed Western blotting in the soma

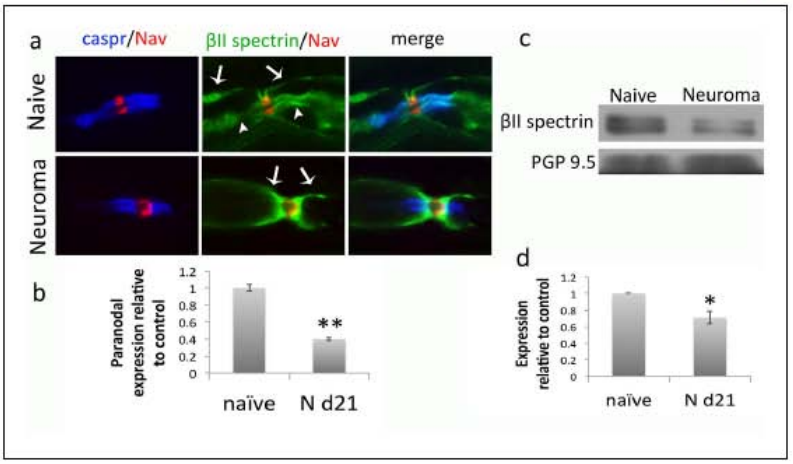

Figure 5. βII spectrin expression in naïve and neuroma nerves. (a) Representative sections of longitudinal nerves immunostained with βII spectrin in green, a panNav antibody in red (to identify the node), and caspr in blue (to identify the paranode). βII spectrin is expressed both in the surface of Schwann cells (arrows) and in the axon at the paranodal and juxtaparanodal region (arrow heads) in naïve nerves. At 21 days after axotomy (neuroma), βII spectrin can be only seen in the Schwann cell (arrows) but not in the axonal domains. (b) Quantification of βII spectrin immunofluorescence in the paranode (identified by caspr staining) showing a significant reduction in neuroma versus naïve (immunofluorescence normalised to naïve: 0.4 ± 0.02, p<0.001, t-test, n = 50–83 heminodes). (c) Western blots showing expression of βII spectrin in the DRG of naïve and day 21 neuroma. (d) Quantification of WBs. Expression of βII spectrin in the DRG was reduced by 30% after nerve injury. PGP9.5 was used as a loading control (expression relative to naïve: 0.7 ± 0.08, p = 0.04, t-test). **p<0.001, *p<0.05. Error bars denote SEM.

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of sensory neurons (dorsal root ganglia- DRG) and observed a 30% decrease following nerve injury compared to naive (expression relative to naïve: 0.7 ± 0.08, p = 0.04, t-test, n = 4, ***Figure 5c–d***).

At day 21 after injury, in marked contrast with the naïve axons, very few of the nodes at the neuroma site (day 21) showed Kv1.2 immunostaining (8.3 ± 0.8% in neuroma vs. 86.1 ± 4.4% in naive, n = 4 animals per group, 25 nodes per animal p<0.001 t-test). Conversely, most of the nodes at the neuroma site (day 21) showed intense Kv1.4 immunostaining (73.3 ± 12% in injured vs. 5.5 ± 4 in naive, n = 4 animals per group, 30 nodes per animal p<0.001 t-test) and Kv1.6 immunostaining (66.6 ± 14% vs. in injured vs. none in naive, n= 4 animals per group, 30 nodes per animal p<0.001 t-test) (***Figure 1***). (Note that naïve nerves were comparable in terms of quantification to nerves from sham-operated animals [p = 0.48]).

We used Western blotting to quantify the expression of the different α subunits and found that Kv1.1 and Kv1.2 expression were significantly reduced at days 7 and 21 following nerve injury, while Kv1.4 and Kv1.6 were significantly upregulated at the neuroma site (***Figure 1***). We looked at Kv1 channel expression in the DRG using IHC and we observed that Kv1.2 expression is reduced after injury, while Kv1.4 and Kv1.6 expression remains unchanged (***Figure 6a***) (this is at the DRG soma, although expression could be seen to increase within paranodes/juxtaparanodes after injury). We also quantified protein expression within the DRG over the same time course using Western blot analysis. The expression of Kv1.2 was significantly reduced following nerve injury (***Figure 6b–e***) consistent with previous findings, (***Everill et al., 1998***; ***Ishikawa et al., 1999***; ***Kim et al., 2002***; ***Yang et al., 2004***), and there was a trend for a reduction in Kv1.1 although this did not reach significance. The expression of Kv1.4 and 1.6 within the DRG did not significantly change following injury

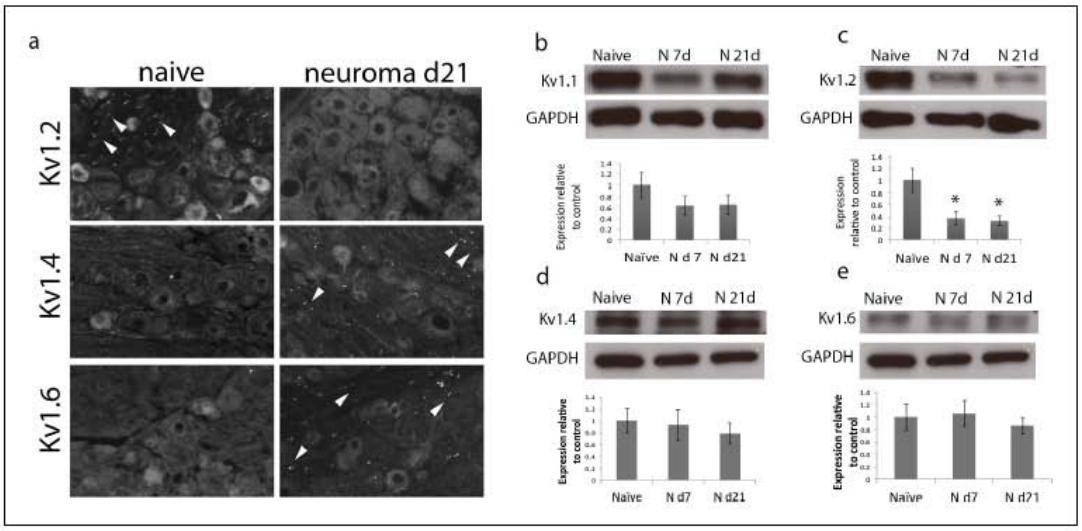

Figure 6. Expression of Kv1 channels in the DRG in the naïve state, 7 and 21 days after axotomy (neuroma). (**a**) Representative sections of naive and neuroma day 21 DRG immunostained with Kv1.2, Kv1.4, and Kv1.6. Note that Kv1.2 expression in DRG cells and axonal juxtaparanodes (arrow heads) is reduced after injury, while Kv1.4 and Kv1.6 expression remains unchanged in DRG cells, and it is present in axonal juxtaparanodes (arrow heads) after injury. In each panel (**b–e**), a representative blot is shown for each time point with GAPDH as a loading control. Quantification of 6 animals per condition is shown below (**b,d,e**) Expression of Kv1.1, Kv1.4 and Kv1.6 within the DRG does not significantly change after axotomy. (**c**) Kv1.2 expression is significantly decreased after axotomy. (*p<0.05, one Way ANOVA, n = 6 per group).

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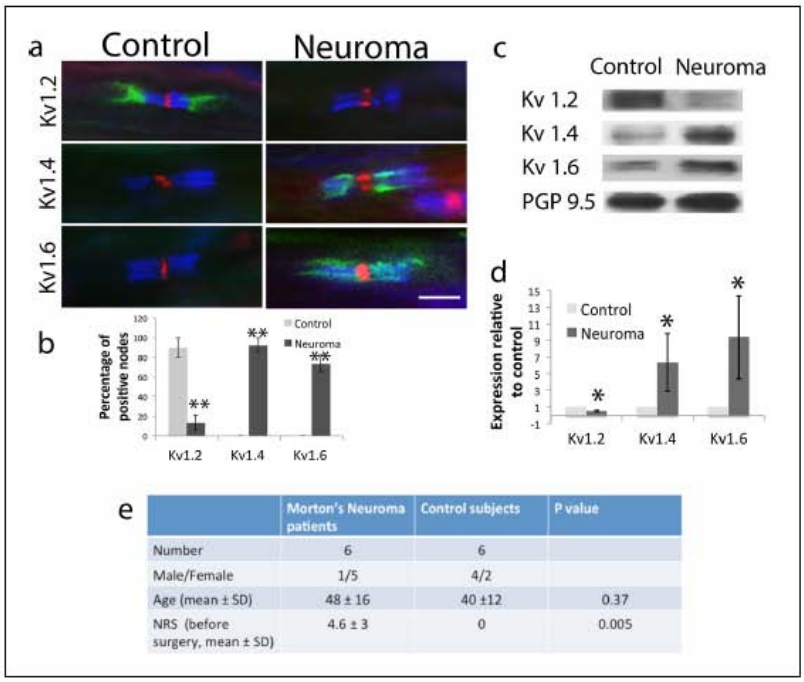
(one Way ANOVA, n = 6 per group) suggesting that increased expression within the juxtaparanode and paranode at the neuroma site is a likely consequence of altered trafficking of these proteins rather than global changes in expression.

We next looked into human nerve tissue to see if these changes were relevant to patients with neuropathic pain We collected 6 control samples (from subjects having their sural nerves removed to use as a bridge for hand reconstructive surgery) and 6 samples obtained from patients undergoing removal of Morton's neuroma (interdigital nerve entrapment neuropathy). IHC (n = 3 per group, 8–10 nodes per patient) showed that only Kv1.2 is expressed in the juxtaparanode of healthy subjects (90 ± 10% of nodes were Kv1.2 positive) with absent Kv1.4 and 1.6 staining as observed in the rat. However, in neuroma Kv1.2 expression in the juxtaparanode was minimal (13.3 ± 8.1%) whilst Kv1.4 and Kv1.6 were expressed in most of the nodes (92.5 ± 7.4% for Kv1.4; 73.5 ± 8.8 for Kv1.6) (***Figure 7a–b***). We used western blotting to quantify Kv1 channels proteins in the nerves of the patients (n = 6 per group) and found that Kv1.2 expression was significantly decreased in neuroma compared to control nerve (to 0.48 ± 0.1 of the control, Mann-Whitney U-Test, p 0.005). In contrast, expression of Kv1.4 and Kv1.6 were significantly increased in neuroma compared to controls (to 6.3 ± 3.5, and 9.4 ± 6.6 of the control respectively, Mann-Whitney U-Test p = 0.005 both) (***Figure 7c–d***).

Kv1 channels change their distribution in the nodal regions at sites distant from the injury

We investigated the localisation of Kv1 channels at a site distant from the injury site. To do so, we used a model of L5 spinal nerve transection (SNT) (***Kim and Chung, 1992***) and studied the dorsal



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	Morton's Neuroma patients	Control subjects	P value
Number	6	6	
Male/Female	1/5	4/2	
Age (mean ± SD)	48 ± 16	40 ±12	0.37
NRS (before surgery, mean ± SD)	4.6 ± 3	0	0.005

Figure 7. Kv1 channels expression in the sural nerve of healthy volunteers (control) and from patients with painful Morton neuroma. (**a**) Representative sections of longitudinal nerves immunostained with Kv1 channels in green (Kv1.2, Kv1.4 and Kv1.6 respectively), a panNav antibody in red (to identify the node), and caspr in blue (to identify the paranode). Kv1.2 is expressed in the juxtaparanode in control nerves but it is not present in the injured nerve. Kv1.4 and kv1.6 are not present in control nerve but are expressed in neuroma within the juxtaparanode and encroaching on the paranode nodes. (**b**) Quantification of the percentage of Kv1.2, Kv1.4, and Kv1.6 positive nodes in control and neuroma nodes (n = 3 per group, one way ANOVA). (**c**) Western blots showing expression of Kv1 channels in control and neuroma nerve. (**d**) Quantification of WBs (n = 6 per group, one way ANOVA). Kv1.2 is expressed in the control nerve and down regulated after axotomy, while Kv1.4 and Kv1.6 have a low/null expression in the control nerve and are up-regulated in neuroma. PGP9.5 was used as a loading control. Error bars denote (**e**) Patients and control subjects demographic data. The female/male ratio is different in patients with Morton neuroma and controls (patients with traumatic lesion of the hand) reflecting the F/M ratio of these different conditions. The mean age of patients with Morton neuroma is slightly higher than in control subjects, although it is not significant (t-test). All patients with Morton neuroma presented with pain (mean NRS 4.6), while control presented no pain in the area innervated by the sural nerve (Mann Whitney test). NRS: numerical rate score. Error bars denote SEM. Scale bars = 5 µm, **p<0.001, *p<0.05.

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roots (ie. proximal to the DRG). We used this model instead of the neuroma model to have certainty that all the dorsal root axons studied had their peripheral terminals injured.

In the dorsal roots from naïve animals, the distance between Nav channels staining and the end of caspr was 3.5 ± 0.2 µm, and the distance between Nav channels staining and the start of Kv1.2 staining was 4 ± 0.2 µm, resulting in a small positive difference between these distances (0.5 ± 0.1 µm, n = 4 animals, 25–32 nodes per animal), indicating there was no overlap. Seven days after transection of L5 spinal nerve this distance was still positive (0.24 ± 0.05 µm, n = 4 animals; distance

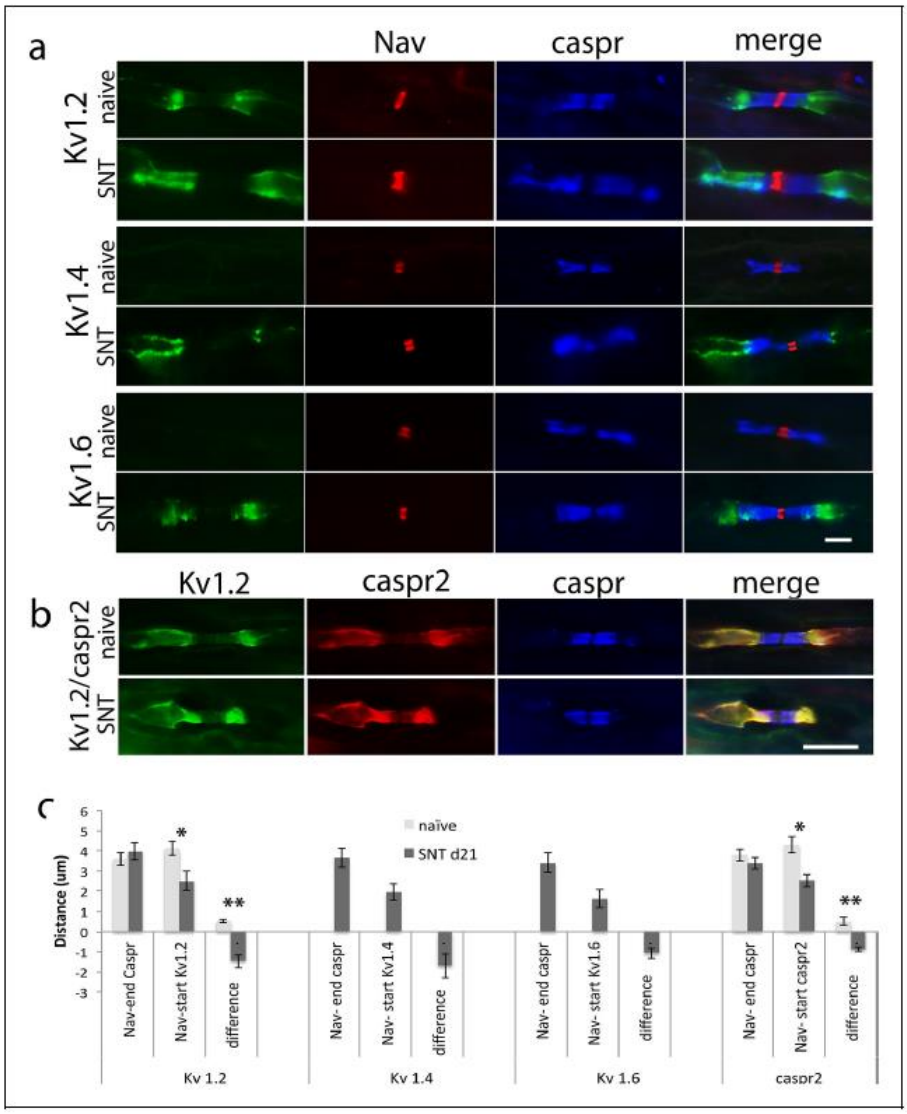

Figure 8. Kv1 channels expression in the dorsal roots of naïve animals and 21 days after spinal nerve transection (SNT). (a) Representative sections of longitudinal dorsal roots immunostained with Kv1 channels in green (Kv1.2, Kv1.4 and Kv1.6, respectively), a panNav antibody in red (to identify the node), and caspr in blue (to identify the paranode). Kv1.2 is expressed only in the juxtaparanode in naïve nerves but after injury it invades the paranode. Kv1.4 and kv1.6 are not present in uninjured nerve but are expressed within the juxtaparanode after nerve injury and also invade the paranode. (b) Re-localization of caspr2 at 21 days after spinal nerve transection (SNT). Representative sections of longitudinal L5 dorsal roots immuno-stained with caspr2 in red, Kv1.2 in green, and caspr in blue. Kv1.2 and caspr2 are expressed in the juxtaparanode in naïve nerves but co-localized with caspr staining at 21 days after injury. (c) Quantification of: distance between the sodium channel staining (Nav) and the end of the caspr staining, distance between the sodium channel staining (Nav) and the start of the Kv1.2/1.4/1.6/caspr2 staining, and difference between these distances. A negative value in this difference represents an overlap of paranodal and juxtaparanodal proteins. Note that the distance between the sodium channel staining (Nav) and the

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end of the caspr staining remains unchanged after nerve injury, while the distance between the sodium channel staining (Nav) and the start of the Kv1.2/1.4/1.6/caspr2 staining is significantly shortened after nerve injury. Kv1.4 and Kv1.6 were absent in naive (n = 5 animals/4 sections per animal, *p<0.05, **p<0.001). Scale bars = 5 µm.

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Nav-end caspr 3.7 ± 0.2 µm, distance Nav-start Kv1.2 3.9 ± 0.1 µm; 30–40 nodes per animal). However, at 21 days after nerve injury, we noted a significant overlap between the end of caspr staining and the start of the Kv1.2 staining (-1.4 ± 0.3 µm, n = 4 animals, 38–40 nodes per animal, one way ANOVA, p<0.001; distance between Nav-end caspr 3.9 ± 0.4 µm, distance between Nav-start Kv1.2 2.5 ± 0.4 µm). We observed this novel localisation of Kv1.2 in the paranode which (in contrast to the neuroma site) is still clearly present after nerve injury within the dorsal root. We also observed novel expression of Kv1.4 and 1.6 in the dorsal root of injured animals, and these were localised to the paranode in addition to the juxtaparanode (the distance between end of caspr and start of Kv1.4 and 1.6 staining was -1.7 ± 0.6 µm and -1.07 ± 0.2 µm respectively; for Kv1.4 distance between Nav-end caspr 3.6 ± 0.4 µm, distance between Nav-start Kv1.4 1.9 ± 0.6 µm; for Kv1.6: distance between Nav-end caspr 3.4 ± 0.4 µm, distance between Nav-start Kv1.6 1.6 ± 0.4 µm n = 4 animals per group, 30–35 nodes per animal ***Figure 8***).

Contactin-associated protein-like 2 (*Caspr2*) is normally localized at the juxtaparanode and associates with K+ channels (***Chiu et al., 2014***). Interestingly, we observed that caspr2 is mobilized into the paranode regions in a similar way to Kv1 channels; the difference between the Nav-caspr staining distance and the Nav-caspr2 distance is minimal in naïve roots (0.5 ± 0.1 µm; distance between Nav-end caspr 3.8 ± 0.3 µm, distance between Nav-start caspr2 4.3 ± 0.4 µm, 30–38 nodes per animal 4 animals), and after SNT, this distance becomes negative (-0.88 ± 0.1 µm, n = 5 animals; distance between Nav-end caspr 3.4 ± 0.3 µm, distance between Nav-start caspr2 2.5 ± 0.3 µm 35–40 nodes per animal p<0.001, t-test) indicating an overlap between caspr and caspr2 immuno-labeling (***Figure 8***).

Effect of Kv1 channels redistribution and change in expression on the incidence of spontaneous activity

Spontaneous activity in naïve axons was present in less than 5% of A-fibres (4.5%). We assessed the effect of blocking the Kv1 channels using α-DTX, a toxin isolated from black and green mamba snakes which is a selective and effective blocker of Kv1-containing oligomers composed of Kv1.1, Kv1.2, or Kv1.6 subunits (***Harvey, 2001***). We applied the toxin at the neuroma site (or, in control animals, acutely cut sciatic nerve stump) and to the L5 DRG and recorded from sensory axons in thin strands dissected from the dorsal root. In the naïve situation (n = 222 neurons, 10 animals, ***Figure 9a***), the incidence of spontaneous activity did not significantly change after α-DTX (5.4 and 9.8% of myelinated afferents when the toxin was applied to nerve stump or L5 DRG, respectively). Two days after transecting the sciatic nerve (n = 291 neurons, 4 animals), spontaneous activity at the injured nerve increased to 22% of myelinated afferents, and it was similar with or without toxin (toxin applied to neuroma 26.1%, toxin applied to the L5 DRG 26%). However, at 7 days after nerve injury (n = 241 neurons, 7 animals) the proportion of spontaneously active afferents had decreased to 6.2%, and application of the toxin now induced a significant increase in proportion of spontaneously firing myelinated afferents (11.2% toxin at the neuroma p = 0.07, and 15.6% toxin to L5 DRG, p = 0.002, chi-square test). At day 21 after injury (n = 237 neurons, 7 animals) spontaneous activity has decreased to baseline levels (2.5% of afferents within the dorsal root), but application of the toxin to both neuroma site or L5 DRG significantly increased the proportion of myelinated afferents demonstrating spontaneous activity (7.2% p = 0.03 and 17.7 ±% p<0.001 respectively, chi-square test) (***Figure 9a***). In summary, we observed an acute increase in spontaneous activity following nerve injury, which was reversed with time. However, at this later time, blockade of Kv1 channels (which had no effect in the naïve state) could reinstate spontaneous activity almost to levels seen acutely

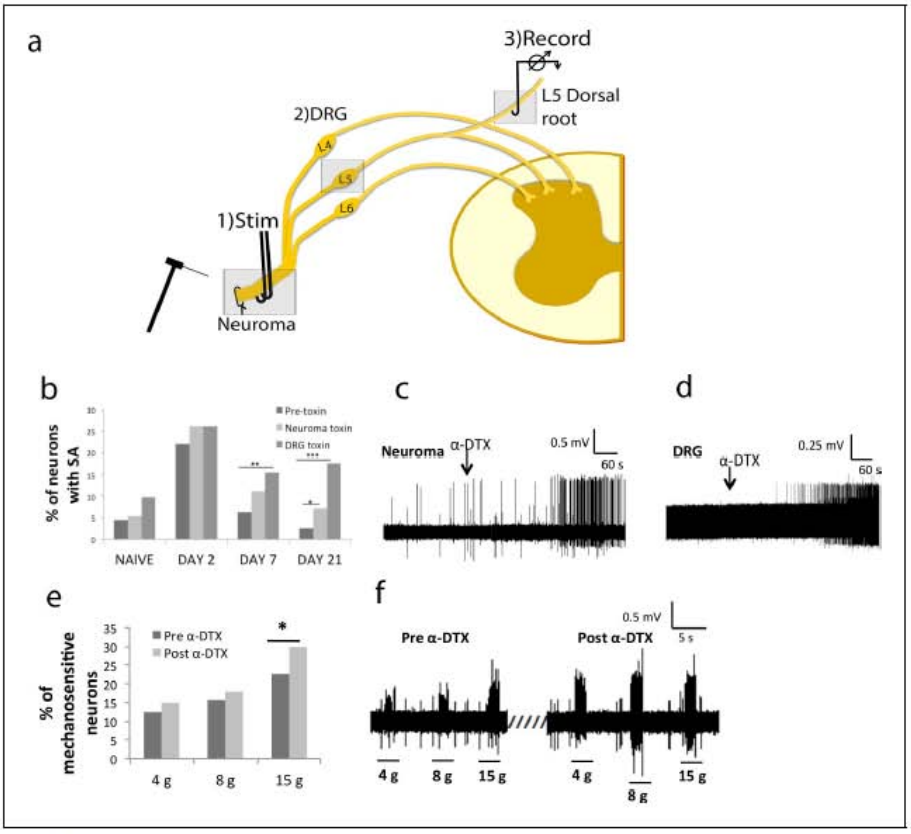

Figure 9. Local application of α-DTX reinstates primary afferent hyperexcitability at later time points following nerve injury. (**a**) Schematic illustration of 3-chamber recording system. 1) Recording chamber, 2) middle chamber, 3) stimulating chamber. The toxin was applied in chambers 1 or 2, respectively. (**b**) Following sciatic nerve transection, there is a large increase in the proportion of primary afferents demonstrating spontaneous activity at day 2, which is suppressed at days 7 and 21 post injury; Local application of α-DTX to the neuroma and L5 DRG at these later time points (days 7 and 21) significantly increases the proportion of afferents, which are spontaneously active (total proportions per group, chi-square tests, *p<0.05, **p<0.01, ***p<0.001) (**c**) neuroma application and (**d**) DRG application of α-DTX. Both recordings were carried out 21 days post-surgery. (**e**) In the presence of α-DTX, significantly more neurons respond to mechanical stimulation at the neuroma site using a 15 g von Frey filament (*p<0.05; total proportions per group, chi-square tests, all neuroma day 21). (**f**) Representative traces showing greater responsiveness to mechanical stimulation with von Frey filaments after local α-DTX application.

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The following source data is available for figure 9:

Source data 1. Source data for ***Figure 9***.

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after nerve injury. Therefore, Kv1 channels appear to have a role in the recovery from increased excitability following nerve injury (***Figure 9b–d***).

Effect of Kv1 channel redistribution and change in expression on mechanical sensitivity

Because Kv1 channels have been shown to have a crucial role in mechano-sensitivity (***Hao et al., 2013***), we tested their role in hypersensitivity following nerve injury. In the presence of α-DTX,

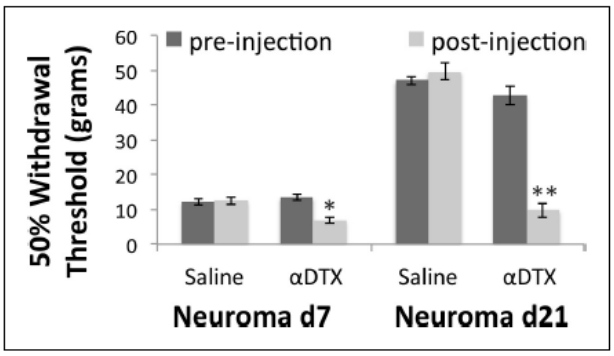

Figure 10. Mechanical hypersensitivity is restored by blocking Kv1 channels. Mechanical withdrawal thresholds were assessed by applying a range of Von Frey hairs to the skin over the neuroma site (labelled with a suture). Animals were randomised to receive either subcutaneous αDTX or saline 30 min before testing. Hypersensitivity after nerve injury is very pronounced until day 7, when it slowly starts recovering. At this time point, perineuromal application of αDTX reversed this early recovery. At 3 weeks after nerve injury hypersensitivity is much recovered and perineuromal injection of αDTX restored mechanical hypersensitivity to levels seen acutely after injury (RM two way ANOVA, *p<0.05, **p<0.001).

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The following source data is available for figure 10:

Source data 1. Source data for ***Figure 10***.

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significantly more neurons responded to mechanical stimulation using a 15g von Frey filament at the neuroma site at day 21 (with 4 g: pre 17.9% post 21.8%; with 8 g: pre 22.1% post 26%; with 15 g: pre 24.7%, post 30.5%, p = 0.006, chi-square test; n = 259 neurons; ***Figure 9e–f***).

Kv1 channels are responsible for the partial recovery of mechanical hypersensitivity seen chronically after nerve injury

We subsequently used behavioural measures to examine mechanical sensitivity after nerve injury. For this purpose, we used the sciatic neuroma model used before in which the sciatic nerve of rats is transected and the proximal end was sutured superficially below the skin on the animal's leg. We applied von Frey filaments of increasing forces to the site of the skin covering the neuroma. To test the role of Kv1 channels on hypersensitivity, we applied α DTX subcutaneously at the site of neuroma. At baseline, the withdrawal threshold was high and did not change with the application of α DTX (vehicle: 142.9 ± 13 g, toxin 150 ± 17.7 g, n = 9 animals per group). Three days after injury, this threshold dropped to 8.2 ± 0.6 g and was not changed by applying the toxin (9.7 ± 1.1 g) (n = 9 animals per group). However, with time this threshold began to normalise reaching 12.9 ± 0.6 g at 7 days (n = 8) and 44.8 ± 1.6 g at 21 days (n = 8). When we injected αDTX, this recovery was not seen and thresholds stayed low (day 7: 6.9 ± 0.9 g, p = 0.003, n = 9; day 21: 13 ± 2.1 g, p<0.001, n = 7, RM two way ANOVA ***Figure 10***).

The time point when the mechanical hypersensitivity began to recover in injured animals coincides with the time when Kv1.1 and Kv1.2 are reduced but Kv1.4 and Kv1.6 are being expressed. α-DTX is a selective blocker for Kv1.1, Kv1.2 and Kv1.6 but has little activity against Kv1.4. CP 339818 hydrochloride however, which is a selective blocker of Kv1.3 and Kv1.4 (***Nguyen et al., 1996***), did not have any effect on mechanical hypersensitivity at early or late time-points post injury suggesting that Kv1.4 is dispensable for the suppression of hyperexcitability. (baselines: saline 133 ± 21 g, CP339818 164 ± 38 g; neuroma day 3: saline 51 ± 2 g, CP339818 44 ± 5 g; neuroma day 7: saline 41 ± 3 g, CP339818 39 ± 4 g; neuroma day 21: 73 ± 13 g, CP339818 91 ± 4 g, RM two way ANOVA, p>0.05, n = 8–7 for saline at day 21).

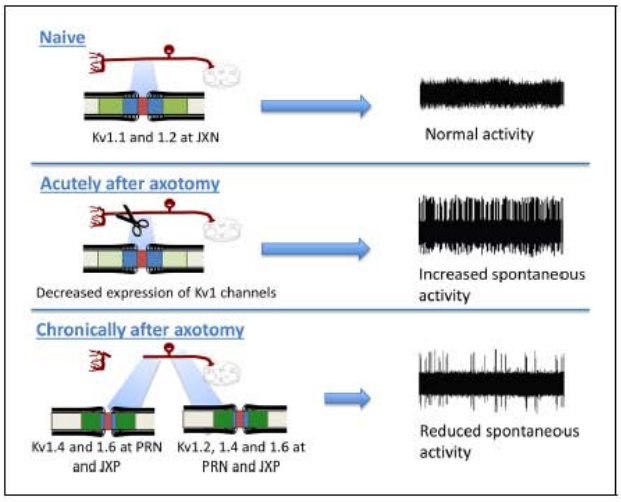

Figure 11. Schematic illustration of the changes in Kv1 channels subunit composition and distribution within the nodal complex and the relationship to hyperexcitability. In the naïve state, Kv1 channels (Kv1.1 and 1.2 shown in green) are localised to the juxtaparanode and separated from voltage-gated sodium channels at the node (red) by the paranode (blue). Acutely following axotomy myelinated primary afferents show a marked increase in spontaneous activity as a consequence of complex changes in increased pro-excitatory drive (for instance from voltage-gated sodium channels) as well as reduced 'brakes' on excitability At later stages within the neuroma, although there is less expression of Kv1.1 and Kv1.2, the expression of Kv1.4 and 1.6 increases. Remote from the injury within the dorsal root expression of Kv1.1 and 1.2 is maintained and the expression of Kv1.4 and Kv1.4 also increases. Furthermore Kv1 channels are redistributed to the paranode as well as being expressed within the juxtaparanode. These changes are associated with a suppression of hyperexcitability.
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Discussion

We have focussed on the distribution of Kv1 channels within the axolemma of myelinated axons in rodent models of neuropathic pain as well as in human neuroma tissue. We show that both at the site of injury (using the neuroma model) and within the dorsal root (remote from the injury site) that there is a major change in the α-subunit composition of Kv1 channels (with increased expression of Kv1.4 and 1.6) and furthermore that Kv1 channels are no longer restricted to the juxtaparanode but also re-distribute to the paranode following injury. Blockade of these ion channels using α-DTX reveals that following axonal injury Kv1 channels act to suppress axonal hyperexcitability and hence hypersensitivity to sensory stimuli (***Figure 11***).

We initially examined the distribution of Kv1 channels within the juxtaparanode of axons within the neuroma at the the injury site. Following traumatic nerve injury there is an initial inflammatory response, demyelination and axonal die-back within the proximal nerve stump. This is followed by axonal sprouting (***Fried and Devor, 1988***; ***DeFelipe, 1991***) (which is often misdirected) as well as proliferation of connective tissue and glial cells. This can lead to a region of nerve swelling, a neuroma, the development of which is well documented in patients (***Scadding, 1981***; ***DeFelipe, 1991***; ***Young, 1942***). Nerve injury is often associated with ongoing pain, dysaesthesia and evoked pain; many patients report that touching the skin overlying or compression of the neuroma site can elicit pain and dysaesthesia (***Nikolajsen et al., 2010***). Unfortunately in many patients, the treatment of neuropathic pain remains inadequate (***Finnerup et al., 2015***) and is associated with significant disability (***Stokvis et al., 2010***). Neuroma pain can be modelled in the rodent using a modified version of the tibial transposition neuroma model (***Dorsi et al., 2008***).

Altered distribution of Kv1 channels in nodal complexes of the neuroma and dorsal root

It has previously been documented that within a neuroma nodes of Ranvier become disorganised (**Levinson et al., 2012**) and we confirm that here with the majority nodes showing abnormalities such as being elongated, split, heminodes or showing Na_v in the absence of caspr. Voltage-gated sodium channels are known to accumulate particularly within axon tips and on denuded axons of the neuroma (**England et al., 1996**). Much less is known regarding the distribution of Kv1 channels changes within the neuroma. This is an important issue as such channels could potentially act as 'brakes' on excitability. At an early time point after injury, we found that Kv1.2 is no longer confined to the juxtaparanode but extended into the paranode. As this could be only a reflection of direct injury, we also looked into a site in the injured nerve 1 cm proximal to the neuroma and found similar changes in distribution, suggesting this is likely to reflect widespread changes within the axon/axoglial signalling (further supported by changes within the dorsal root and discussed below). At a later time-point following injury, we found a striking reduction in the expression of Kv1.1 and 1.2 which are normally localised to the juxtaparanode. In contrast Kv1.4 and 1.6 which are present at a low level in the naïve state are up-regulated and are present both in the paranode and the juxtaparanode. We found broadly similar changes in rodent and human neuroma. This altered expression and localisation is likely to partially reflect the altered relationship between axons and myelinating Schwann cells. Within the neuroma new nodes of Ranvier will be formed as a consequence of myelination of new axon sprouts and remyelination of denuded axons (**Dorsi et al., 2008**; **Dyck et al., 1985**). During developmental myelination and remyelination following primary demyelination (in which the axon remains intact) Kv1.1 and 1.2 can at early time points be observed in other regions apart from the juxtaparanode (at the node of Ranvier and the paranode) before being restricted to the juxtaparanode as the nodal complex matures (**Rasband et al., 1998**; **Vabnick et al., 1999**; **Poliak et al., 2001**). Kv1.4 and 1.6 expression has not to our knowledge been examined during myelination/remyelination.

We also studied the dorsal root, which is remote from the injury site to establish whether there were changes in Kv1 channels composition of the juxtaparanode that reflect the general response of the axon to injury rather than local effects such as inflammation and remyelination at the injury site. We also found striking changes within the nodal complex of sensory axons within the dorsal root. Kv1.2 was no longer down-regulated as had been noted at the neuroma site but its localisation changed following injury: They could be observed in the paranode as well as the juxtaparanode. In the naïve state, very little Kv1.4 and 1.6 expression was noted in the juxtaparanode of the dorsal roots. This is in agreement with previous studies that reported a low frequency of Kv1.4 immunoreactive juxtaparanodes (**Everill et al., 1998**) and no expression of Kv1.6 (**Utsunomiya et al., 2008**). We found however that nerve transection led to markedly increased expression of these α-subunits and they were localised both to juxtaparanode and paranode.

Factors governing localisation of Kv1 channels to axonal domains in the naïve and injured state

What factors are responsible for the altered distribution of Kv1 channels within the juxtaparanode? We used ultrastructural examination of the juxtaparanode in the dorsal root to examine whether structural changes within the paranode could explain the movement of Kv1 channels into this region (from which they are normally excluded). The transverse bands are important points of attachment between the axon and the paranodal loops of the Schwann cell. These axoglial septate-like junctions are formed by the interaction of caspr (**Bhat et al., 2001**) and contactin (**Boyle et al., 2001**) on the axolemma binding with the 155Kd isoform of Neurofascin expressed on the Schwann cell paranodal loops (**Tait et al., 2000**). These junctions act as diffusion barriers between the nodal and juxtaparanodal membrane. Mice lacking caspr (**Bhat et al., 2001**), contactin (**Boyle et al., 2001**) or NF155 (**Pillai et al., 2009**; **Sherman et al., 2005**) have absent transverse bands, increased distance between the axon and Schwann cell membrane, disorganisation of the paranodal loops and probably as a consequence of the loss of this lateral diffusion barrier Kv1 channels are noted to extend into the paranode. Similarly in mice lacking ceramide galactosyl transferase in which all-putative adhesion components of the paranodal junction are lacking, Kv1.2 and caspr2 are also mis-localised to the paranodes (**Poliak et al., 2001**; **2003**). On ultrastructural examination of the paranodes within the

sciatic nerve following injury, we did not see major structural changes and there was no increase in the distance between the axon and the Schwann cell membrane at the site of attachment of the paranodal loops. We noted an increase in the maximum distance between paranodal loops, which is unlikely to alter the ability of molecules to passively diffuse between the membrane domains of the juxtaparanode and paranode (however it will increase the diameter of the helical pathway between paranodal loops connecting the extracellular space to the axonal internode. One potential consequence of which would be reduced passive resistance to current flow between the node and voltage-gated potassium channel (VGKC) in the juxtaparanode and paranode, which could then have a greater influence on nodal excitability (**Shroff et al., 2011**). A recent publication has demonstrated that in mice lacking βII spectrin expression in axons Kv1 channels were no longer restricted to the juxtaparanode but could also be observed in the paranode even though the structural integrity of axoglial junctions was intact (**Zhang et al., 2013**). βII spectrin contributes to the sub-membranous cytoskeleton of the axon-linking membrane proteins to actin and appears to act as a barrier limiting the lateral diffusion of membrane proteins. We found that paranodal expression of βII spectrin was reduced following axotomy and a reduction in this sub-membranous barrier is compatible with the lateral movement of Kv1 channels into this region that we observed. As well as Kv1 channels we also see caspr2 overlapping with paranodal markers following nerve injury and again such paranodal localisation of caspr2 was also reported in mice in which axonal βII spectrin is conditionally ablated. Caspr2 complexes with and is important for the correct localisation of Kv1 channels (**Poliak et al., 1999**; **2003**) suggesting that this whole protein complex is mis-localised following nerve injury. Although loss of a sub-membranous barrier to diffusion of the VGKC-complex is one explanation for their paranodal localisation, we do not yet have a full understanding of the regulation of Kv1 channels trafficking. Phosphorylation events may have a role to play as Kv1.2 can undergo phosphorylation, which impacts on surface expression/localisation (**Gu and Gu, 2011**; **Yang et al., 2007**).

The effect of altered Kv1 channels subunit composition and localisation on axonal excitability and neuropathic pain

Following axonal injury sensory axons become hyper-excitable and this is important in driving and maintaining neuropathic pain (**Han et al., 2000**). A recent study showed that myelinated sensory fibres are key in maintaining mechanical allodynia in several neuropathic pain models (**Xu et al., 2015**). Spontaneous activity and mechanical stimulus evoked activity has been recorded in myelinated afferents innervating neuroma using microneurography (**Nyström and Hagbarth, 1981**). The role of Kv1 channels has mainly focussed on their importance in suppressing excitability at the soma rather than the axon following injury. The expression of a number of α Kv1 channels sub-units has been documented to decrease following peripheral axotomy including Kv1.1, 1.2 (**Everill et al., 1998**; **Hao et al., 2013**; **Ishikawa et al., 1999**; **Kim et al., 2002**; **Yang et al., 2004**) and in some reports Kv1.4 (we did not see a reduction in Kv1.4 using western blot analysis of DRG lysate following sciatic axotomy, however, this is a less proximal lesion compared to spinal nerve ligation [**Everill et al., 1998**]). Correspondingly, the K currents mediated by such channels are reduced when measured at the soma (**Yang et al., 2004**) both in small and large diameter DRG cells. The focus has therefore been on the loss of K currents within the soma which normally act as a 'break' on excitability, and combined with increased excitatory drive for instance due to the dysfunction of voltage-gated sodium channels and hyperpolarization-activated cyclic nucleotide-gated (HCN) channels this leads to ectopic activity (**Waxman and Zamponi, 2014**). While changes at the soma are undoubtedly important, ectopic impulses also arise along the axon. There has been much less focus on the distribution and function of Kv1 channels within the axon following peripheral nerve injury.

The function of Kv1 channels is critically dependent on their targeting to specific neuronal compartments (**Trimmer, 2015**). The expression of Kv1 channels within the DRG soma and the axon should not be assumed to be the same: For instance the expression of Kv1.6 remains stable within the DRG both at the level of mRNA and protein (see **Kim et al. (2002)**, **Yang et al. (2004)** and our own data) but as we show here expression markedly increases in axons within peripheral nerve and dorsal root. Altered α subunit composition and localisation of Kv1 channels is likely to have functional implications. In the naïve state, the Kv1 channels Kv1.1 and 1.2 are located within the juxtaparanode, below the insulating myelin sheath and at least in peripheral nerves have little functional impact on nodal excitability and conduction (**Poliak et al., 2003**; **Chiu and Ritchie, 1980**; **Sherratt et al., 1980**; **Rasband et al., 1998**). During development when Kv1 channels are observed

in the node and paranode using specific blockers of this K current suggests that these Kv1 channels prevent re-entrant excitation in motor axons (**Vabnick et al., 1999**). Following primary demyelination, the re-distribution of Kv1 channels to the paranode acts to suppress continuous conduction in demyelinated axons (**Rasband et al., 1998**). In certain contexts therefore and especially when Kv1 channels begin to encroach on the paranodal regions there is evidence that these channels can suppress excitability of the axon. As has been previously demonstrated we have found that the rate of ectopic activity within myelinated axons is very high in the first week and then decreases the longer the time elapsed following the initial injury (**Campbell et al., 1988**). Understanding adaptive mechanisms to suppress such hyper-excitability will potentially provide insight as to why in certain patients such mechanisms fail leading to chronic pain states. Over a similar time period as this reduction in spontaneous activity we have observed increased expression of Kv1.4 and 1.6 as well as redistribution of Kv1 channels to the paranode and juxtaparanode domains. Selective inhibition of Kv1 channels with α-DTX reinstates a higher level of ectopic activity, increases mechanosensitivity of afferents innervating the neuroma and on behavioural testing also exacerbates mechanical hypersensitivity, which had begun to normalise at 3 weeks post injury. α-DTX blocks Kv1.1, Kv1.2 and Kv1.6. As expression of Kv1.1 and 1.2 are decreased while Kv1.6 is increased, most probably the effect seen with the toxin is through Kv1.6. Selective inhibition of Kv1.4 did not recapitulate these events emphasising the role of Kv1.6 (and subunits with which it complexes) in suppressing hyperexcitability. Neuronal Kv1 proteins form heterotetramerization of α subunits, which also associate with auxiliary Kvβ subunits (**Jan and Jan, 2012**), adding complexity in ascribing function to individual α subunits. α subunits confer particular pharmacological and biophysical properties on these channels and in addition there may be interactions between subunits. For instance Kv1.4, usually shows N-type rapid inactivation through an N-terminal inactivation ball however this can be over-ridden if associated with a Kv1.6 α subunit (**Roeper et al., 1998**), due to its NIP (N-type inactivation prevention) domain.

In conclusion, we have found major changes in Kv1 channels subunit composition and distribution within the axolemma of myelinated axons following traumatic nerve injury. In contrast to the soma in which Kv1 channels expression is reduced this increased availability of Kv1 channels within the paranodes and altered subunit composition appears to fulfil an adaptive role in suppressing excessive excitability in myelinated afferents.

Materials and methods

Animals and surgery

Adult male Sprague-Dawley rats were used in accordance with UK Home Office and Pontificia Universidad Catolica's regulations (animals in the UK were purchased from Charles-River UK, animals from Chile were purchased onsite). Rats were group housed and placed on a 12 hr-light 12 hr-dark cycles. Two different nerve injury models were used: the neuroma model and the L5 spinal nerve transection (SNT) model. The neuroma model of neuropathic pain was based on the TNT model (**Dorsi et al., 2008**), but performed with some modifications. Briefly, the sciatic nerve was dissected free of adjacent tissue, ligated with a suture, and cut proximal to its bifurcation. The needle from the suture was passed through a subcutaneous tunnel to the lateral aspect of the hindlimb where it was pushed through the skin. The nerve was drawn into the tunnel until the ligature is adjacent to the skin. The suture was cut, and the incision closed. The suture tied to the distal end of the sciatic nerve was visible through the skin and served as the target for mechanical stimuli. An analogous site served as the target on the contralateral hindlimb. For the L5 SNT model (**Kim and Chung, 1992**), one-third of the L6 transverse process was removed and the L5 spinal nerve was identified and dissected free from the adjacent L4 spinal nerve and then tightly ligated using 6–0 silk and then transected distally to the suture. Sham-operated animals served as a control. We used these two different models as the neuroma model is the most adequate for performing behavioural tests as the injured nerve can be directly stimulated, while the L5 SNT model has the advantage that it gives certainty that all the dorsal root axons studied had their peripheral terminals injured. For both models animals were deeply anaesthetised with a mix of isofluorane and oxygen. Postoperative analgesia was given for the first 5 days postop (tramadol 50 mg/kg/day p.o). Animals were checked every day after surgery to check for self-mutilation behaviour (autotomy), which prompted us to sacrifice the

Table 1. Different antibodies used in the study.

Antibody	Concentration used IHC WB	Company
Rabbit anti Pan voltage gated sodium channel (Cat No. S6936)	1:1000	Sigma-Aldrich
Mouse anti Kv1.2 (K14/16.2)	1:100 1:500	UC Davis/NIH NeuroMab Facility
Mouse anti Kv1.1 (K36/15.1)	1:100 1:200	UC Davis/NIH NeuroMab Facility
Mouse anti Kv1.4 (K13/31)	1:100 1:200	UC Davis/NIH NeuroMab Facility
Mouse anti Kv1.6 (K19/36)	1:100 1:500	UC Davis/NIH NeuroMab Facility
Guinea Pig anti Caspr	1:1000 1:1000	From Dr Manzoor Bhat - UT Health Science Center San Antonio (***Bhat et al., 2001***)
Rabbit anti Caspr2 (ab105581)	1:500 1:400	Abcam
Rabbit anti Pan Neurofascin	1:500	Gift from Prof Peter Brophy-University of Edinburgh (***Pomicter et al., 2010***)
Mouse anti βII spectrin (Clone 42)	1:500 1:1000	BD Bioscience
GAPDH	1:10000	Abcam
PGP 9.5	1:5000	Ultraclone

IHC: Immunohistochemistry; WB: Western Blot analysis.

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animal. Calculation of the sample size needed was done for each experiment as described below. Experimental protocols were reviewed and approved by 'Coordinación de Ética, Bioética y Seguridad de las investigaciones UC' (experiments done in Chile) and were performed in accordance with the UK Home Office regulations (experiments done in the UK). We report this study in compliance with the ARRIVE guidelines (***Kilkenny et al., 2010***) (20 points checklist).

Patients and controls

The study was conducted at Hospital Clinico UC-Christus in Santiago, Chile. Morton's neuroma patients that were due surgery for resection of painful neuromas were recruited for donating a small sample of the tissue resected during surgery. Control samples were obtained from subjects undergoing hand reconstructive surgery in where the sural nerve is harvested and used as a bridge to connect disrupted ends of motor nerves in the hand. A small sample for these healthy sural nerves was collected to use as control in this study. We used the Numeric Rating Scale (NRS; which is a self-reporting scale where 0 is no pain and 10 is the worst imaginable pain) to assess for pain before surgery. Informed consent was obtained from all subjects before surgery. The study protocol was assessed and approved by the Ethics Scientific Committee of the School of Medicine Pontificia Universidad Catolica de Chile (reference number 14–389). The sample sizes were calculated using a power of 80% and an α error of 0.05%, assuming a 2 times increase or decrease in Kv channel expression with a variance of 0.6 from the mean, which resulted in a sample needed of 3 patients per group.

Histology

After a defined survival time (7 and 21 days), animals were terminally anaesthetized with pentobarbital and transcardially perfused with 0.9% heparinised saline. The L5 DRG, the L5 spinal nerve, and the sciatic nerve were removed. We dissected the sciatic nerve free from connective tissue and collected 5 mm from the site of the neuroma and 5 mm from a site 1 cm proximal. Tissue for immunohistochemistry was post fixed in 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB) for 30 min and cryoprotected in 20% sucrose for 3 days. Tissue obtained from patients was fixed immediately after resection in 4% PFA for 30 min and then cryoprotected in 20% sucrose for 3 days. The samples were embedded in OCT, cryostat cut (8 µm) and thaw-mounted onto glass slides. Sections were pre-incubated in buffer (PBS, pH 7.4, containing 0.2% Triton X-100 and 0.1% sodium azide)

containing 10% normal donkey serum for 30 min and then incubated with primary antibodies overnight at room temperature. Primary antibodies used are shown in **Table 1**. Following primary antibody incubation, sections were washed and incubated for 2 hr with secondary antibody solution (donkey anti-rabbit Cy3 1:400; goat anti guinea pig AMCA 1:100, donkey anti mouse FITC 1:400; all from Stratech, UK). Slides were washed with PBS, cover-slipped with Vectashield mounting medium (Vector Laboratories, UK) and visualised under a Zeiss Axioplan 2 fluorescent microscope (Zeiss, UK). All quantification of different IHC parameters was done with the investigator blinded to the identity of the group to which the animals belonged. Nodal quantification was done by assessing on average 31 nodes per animal, and using 4–5 animals per condition. For quantification of βII spectrin the intensity of the immunofluorescence of the axonal paranodal area (identified by caspr staining) was measured and the background of each section was subtracted. Then, measurements were normalised against the mean of the controls (naïve axons). The sample sizes were calculated using a power of 80% and an α error of 0.05%, assuming a 2 times increase or decrease in expression with a variance of 0.5 from the mean, which resulted in a sample needed of 4 animals per group.

We quantified sodium channel clusters following the following criteria: (1) typical nodes were nodes where the Nav channels fill the gap at the node of Ranvier as identified by the paranodal staining of caspr on both sides of the node, (2) split nodes were nodes that had two distinct Nav channels accumulations, separated by a gap in the Nav channel staining within the same fibre and with each Nav channels accumulation flanked on one side with caspr staining, or (3) heminodes were nodes where the caspr staining located on only one side of a contiguous Nav channel accumulation, (4) while those Nav channel accumulations lacked an association with caspr were classified as 'naked' accumulations.

Western blot analysis

Tissue was collected, quickly frozen in liquid nitrogen and was homogenized in NP40 lysis buffer (20 mM Tris, pH 8, 137 mM NaCl, 10% glycerol, 1% NP-40, 2 mM EDTA), 20 µM leupeptin, 5 mM sodium fluoride, 1 mM sodium orthovanadate, 1 mM PMSF and protease inhibitor cocktail). The lysate were spun at 13,000 rpm at 4°C for 15 min and the protein concentration of supernatant was determined using a BCA Protein Assay kit (Thermo Scientific). 50 µg of each sample was separated using 8% or 10% SDS-PAGE, and transferred to nitrocellulose membranes. Membranes were then blocked in 10% skimmed milk in PBS-T (PBS+ 0.1% Tween 20) for 1 hr at room temperature. Membranes were incubated with primary antibody (anti mouse Kv1.1, Kv1.2, Kv1.4, Kv1.6, GAPDH, PGP9.5 and anti-rabbit Caspr2 as shown in **Table 1**), diluted in PBS-T at 4°C overnight. After washing with PBS-T for 6 times and5 min each time, membranes were incubated with sheep anti-mouse or donkey anti rabbit HRP-conjugated secondary antibody (1:10,000–1:20,000; ECL, GE Healthcare, Amersham, UK) at room temperature for 1 hr. After several PBS-T washes as described above membranes were revealed using ECL-prime reagent (GE Healthcare) for 5 min for detection by autoradiography.

For WB of Kv1.4 and Kv1.6 in rat tissue (sciatic nerve and DRG) the membranes were cut in three pieces; the top piece was probed with Kv1.4 antibody, the middle one was probed with Kv1.6 antibody and the bottom one probed with GAPDH antibody. For WB of Kv1.1 and for Kv1.2 the membranes were cut in 2 pieces: the top one was probed with either Kv1.1 or Kv1.2 antibody and the bottom one was probed with GAPDH antibody. The 2 or 3 pieces of the membranes were lined up as a single membrane before exposing it to the film so that the molecular weight can be calculated by measuring the running distance of the molecular weight marker and the target bands. This could be done as the bands labelled by the antibodies have quite different molecular weights. This allowed us to optimize the use of the tissue obtained from animals and reduce the number of animals needed (in accordance with our obligations under animal licensing procedures).

Quantification and analysis

For Western Blots analysis, films were scanned with Cannon Scanner (LiDE 210), and the intensity of specific bands was quantified using Quantity One software (Bio-Rad). The same size rectangle was drawn around each band to measure intensity, and the background was subtracted. Target band detected was normalized against loading control GAPDH or PGP9.5 correspondingly for analysis. The sample sizes were calculated using a power of 80% and an α error of 0.05%, assuming a 2 times

increase or decrease in expression with a variance of 0.5 from the mean, which resulted in a sample needed of 4 animals per group (we used 6 animals per group in case we had to put any animal down due to autotomy).

Electron microscopy

Sciatic nerves were dissected at the site of the neuroma and were processed for resin embedding as previously described (Huang et al). Briefly nerves were post fixed in 3% glutaraldehyde at 4°C overnight, washed in 0.1 M PB, osmicated, dehydrated, and embedded in epoxy resin (TAAB Embedding Materials, UK). Longitudinal sections 1 µm thick were cut on a microtome and stained with toludine blue before being examined on a light microscope. Ultrathin sections were cut on an ultramicrotome and stained with lead uranyl acetate. Sections were mounted on unsupported 100 mesh grids. Sections were visualised on a PHILIPS TECNAI 12 BIOTWIN transmission electron microscope at the Unidad de microscopia avanzada, Pontificia Universidad Catolica de Chile. We measured the diameter of the axons at the site of the node, the maximal and minimal distance between interloops, the distance between the glia and the axon, the number of detached loops, and the number of everted loops using Image J (NIH, USA) and a 135000x magnification. We quantified between 8 and 14 nodes per animal, and we used 5 animals per condition (sample sizes were calculated using a power of 80% and an α error of 0.05%, assuming a change in distance of 50% with a variance of 0.4, which resulted in a sample needed of 4 animals per group, however due to the difficulty in the technique we included one more animals in each group). The investigator was blinded to the treatment group of each specimen, however, this was sometimes difficult to conceal as the anatomy in the injured nerves was much more disrupted than in naive nerves.

In vivo electrophysiological recording

Recordings were performed under anaesthesia (urethane, 1.5 g/Kg, i.p.) on naïve rats (n = 10, 222 neurons), or after sciatic nerve ligation at 2 days (n = 4, 291 neurons), at 7 days (n = 7, 241 neurons), and at 21 days (n = 7, 237 neurons). A tracheotomy was performed and the L5 dorsal roots and DRGs were exposed via laminectomy. Sciatic nerve neuroma with proximal nerve (5–6 mm long) and contralateral uninjured sciatic nerve were exposed. The contralateral sciatic nerve was acutely cut to disconnect from the periphery just before recording. The entire site was covered in agarose gel and four chambers created by removing blocks of this gel. These were 1) neuroma chamber, containing ipsilateral neuroma and part of sciatic nerve which is subjected to stimulation; 2) acutely cut nerve end chamber, containing contralateral sciatic nerve proximal end; 3) DRGs chamber, containing L5 DRGs from both sides; 4) spinal recording chamber, containing part of L5 dorsal roots from both sides near entry zone to spinal cord. The neuroma chamber and nerve cut end chamber were filled with mineral oil during stimulation, and the oil was replaced with αDTx (100 nM in saline) during toxin application. The DRGs chamber was filled with saline or αDTx/saline solution, and the recording chamber was always filled with mineral oil. The pool temperatures were not controlled, but as animals were warmed using an infrared lamp from the back, the pool was therefore heated, and typically was at 34–35°C. Just before recording, the L5 dorsal root was cut near entry zone, a filament was teased out and hooked up for recording. Each filament was stimulated electrically with increasing current to recruit sequentially each conducting axon in that filament. The conduction velocity of each conducting axon was noted. Thus, the number of functioning axons in each filament was determined (typically, 6–10). Spike discrimination was used to detect the number different axons firing spontaneously in each filament (typically 0–3) during a pre-treatment baseline and under 3 different treatment conditions: 1) no αDTx in any of the chambers; 2) αDTx in neuroma or nerve cut end chamber; 3) αDTx in neuroma or nerve cut end chamber and DRGs chambers. The αDTx was applied for at least 20 min before recording. An independent investigator prepared the drugs individually and labelled them for each animal according to the randomization schedule. Data analysts were blinded as the conditions under which all recording were made.

Signals were amplified with an AC-coupled amplifier (Neurolog NL104A with headstage NL100AK), then high-pass- and low-pass filtered (Neurolog NL125) at 500 Hz and 5 KHz frequencies. The filtered signals were passed through a Humbug 50 Hz noise eliminator (Quest scientific, Vancouver, BC, Canada), further amplified (Neurolog NL 106), fed to an analog-to-digital converter PowerLab, and sampled at 20 KHz with Labchart software (ADinstruments, UK). Stimulation (200 µs

square-wave pulses) was delivered from a stimulus isolator (Neurolog, NL800A). The filter settings used strongly favours recordings from A-fibres and not C-fibres. All the fibres recorded to nerve stimulation conducted in the A fibre range (>2 m/sec). The size of the filaments recorded was also unfavourable for detecting clear single unit C fibre activity.

Three minutes baseline was recorded to examine spontaneous activity. The percentage of spontaneously firing units was calculated as the number of spontaneously active units divided by the number of conducting fibres determined in recruitment recording. The firing rate was calculated as the total number of spikes during recording divided by the time recorded. The mean firing rate per unit was the firing rate divided by the total number of different units recorded in each treatment group.

For the axonal mechanosensitivity experiments (n = 4, 259 neurons), mechanical stimulation was applied to the neuroma using increasing forces of von Frey filaments (4, 8, and 15 g), and the number of distinct spikes (neurons) firing in response were counted following spike discrimination. The total number of conducting axons in each filament was determined by incremental electrical stimulation of the sciatic nerve. The percentage of mechanosensitive units was calculated as the number of different neurons responding by firing action potentials upon mechanical stimulation, divided by the number of conducting fibres (which was determined in the same way as for the spontaneous activity experiments). Axonal mechanosensitivity was assessed before and after toxin application at 21 days after axotomy. Mechanosensitivity experiments were carried out on separate animals to spontaneous activity experiments to ensure that any spontaneous activity encountered was not caused acutely by the repeated mechanical stimulation of the neuroma.

Data was analyzed using software Labchart. Statistics comparing proportions of neurons exhibiting either spontaneous activity or mechanosensitivity were performed using chi-square test with Yates correction. Values were reported as percentages, calculated from the proportions.

Assessments of mechanical sensitivity

Mechanical withdrawal thresholds were assessed by applying a range of Von Frey hairs (Somedic, Sweden) to the skin over the neuroma site (labelled with a suture as previously described). Animals were randomised to receive either subcutaneous αDTX (0.5 ml at 100 nM in saline, Alomone, UK) or saline (which was administered locally at the site of the neuroma) using a computer-generated random sequence. The sample sizes were calculated using a power of 80% and an α error of 0.05%, assuming a 60% decrease in withdrawal threshold with a variance of 25% from the mean, which resulted in a sample needed of 7 animals per group. Experimental groups were the following: baseline with vehicle, baseline with toxin; day 3 after surgery with vehicle, day 3 with toxin; day 7 with vehicle, day 7 with toxin; day 21 with vehicle, day 21 with toxin. To reduce the amount of animals of the study the animals that received saline only were used again for the consecutive time-points. The animals that received toxin had to be sacrificed after testing, as the toxin is irreversible. The toxin or saline were injected 30 min before testing. Autotomy after nerve injury (especially neuroma model) appears at around 10 days after injury. Therefore, we allocated 2 extra animals for the saline group, and 2 extra for toxin day 21. We had to sacrifice 1 animal from the saline group at day 6, and 2 animals from the toxin group day 21 (at day 15 and 17 after injury respectively), due to self-mutilating behaviour. For testing, rats were gently restrained using a towel on a table. Calibrated von Frey hairs were applied to the skin covering the neuroma until the fibre bent. Withdrawal of the limb by the animal was recorded as a response. The 50% withdrawal threshold was determined using the up-down method (Dixon, 1980). An independent investigator prepared the drugs individually and labelled them for each animal according to the randomization schedule. Operators and data analysts were blinded throughout the study. The data were distributed normally and the differences between groups was analysed using a 2 way ANOVA repeated measures. Values were reported as mean ± SEM.

This experiment was repeated for testing CP339818 (Kv1.4 blocker; #C-115, 0.5 ml at 300 nM in saline Alomone Labs UK). We randomly allocated 8 animals per group, and we had to put one animal from the saline group down due to autotomy at day 12.

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Ethics

Human subjects: Informed consent, and consent to publish, was obtained from all subjects to collect and analyze nerve samples before surgery. Subjects underwent surgery by indication of their physician and samples were obtained from biological tissue that was otherwise due to be incinerated. The study protocol was assessed and approved by the Ethics Scientific Committee of the School of Medicine Pontificia Universidad Catolica de Chile (reference number 14-389).
Animal experimentation: This study was performed in strict accordance with UK Home Office and Pontificia Universidad Catolica's regulations. Experimental protocols were reviewed and approved by "Coordinación de Ética, Bioética y Seguridad de las investigaciones UC" (experiments done in Chile- Protocol CBB230/2013) and were performed in accordance to the UK Home Office regulations (experiments done in the UK). We report this study in compliance with the ARRIVE guidelines (20 points checklist).

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Research Paper

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Symptom profiles in the painDETECT Questionnaire in patients with peripheral neuropathic pain stratified according to sensory loss in quantitative sensory testing

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Abstract

The painDETECT Questionnaire (PDQ) is commonly used as a screening tool to discriminate between neuropathic pain (NP) and nociceptive pain, based on the self-report of symptoms, including pain qualities, numbness, and pain to touch, cold, or heat. However, there are minimal data about whether the PDQ is differentially sensitive to different sensory phenotypes in NP. The aim of the study was to analyze whether the overall PDQ score or its items reflect phenotypes of sensory loss in NP as determined by quantitative sensory testing. An exploratory analysis in the Innovative Medicines Initiative Europain and Neuropain database was performed. Data records of 336 patients identified with NP were grouped into sensory profiles characterized by (1) no loss of sensation, (2) loss of thermal sensation, (3) loss of mechanical sensation, and (4) loss of thermal and mechanical sensation. painDETECT Questionnaire profiles were analyzed in a 2-factor analysis of variance. Patients with loss of thermal sensation (2 and 4) significantly more often reported *pain evoked by light touch*, and patients with loss of mechanical sensation (3 and 4) significantly more often reported *numbness* and significantly less often *burning sensations* and *pain evoked by light touch*. Although the PDQ was not designed to assess sensory loss, single items reflect thermal and/or mechanical sensory loss at group level, but because of substantial variability, the PDQ does not allow for individual allocation of patients into sensory profiles. It will be useful to develop screening tools according to the current definition of NP.

Keywords: Quantitative sensory testing, German Research Network on Neuropathic Pain, painDETECT, Neuropathic pain, Questionnaire, Polyneuropathy, Peripheral nerve injury

1. Introduction

Neuropathic pain (NP) is defined as pain resulting from a lesion or disease of the somatosensory system[2,37] and can be caused by many clinical etiological entities such as nerve injury, herpes zoster, or polyneuropathy (PNP).[2,29] The identification of probable NP is based on a plausible medical history with the distribution of pain consistent with the innervation territory of the suspected lesioned nerve structure. Furthermore, a clinical examination including the assessment of negative (loss of function) or positive (gain of function) sensory signs is required. The sensory phenotype can be assessed by bedside testing[2,7] or quantitative sensory testing (QST).[1,23] The underlying lesion or disease of the somatosensory system can then be further examined by a diagnostic test, eg, electroneurography, skin biopsy, or corneal confocal microscopy.[15,24,26,37] Loss of sensation implies a dysfunction, lesion, or disease in different types of nerve fibers: loss of

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function of the thickly myelinated Aβ fibers can be demonstrated by clinical testing of the sensation of touch and vibration[27] or tactile and vibration detection threshold (VDT) (eg, in QST). Loss of small fiber (Aδ-fiber and C-fiber) function is characterized by hypoesthesia to thermal stimuli, which can be tested using QST by assessing the cold detection threshold (CDT) and warm detection threshold (WDT) or in bedside testing by cold and warm metallic rollers or water tubes and pins.[26,24] Both types of sensory loss may appear in patients suffering from NP separately, in combination, or might not appear at all. It has been shown that they may be important predictors for the response of patients to certain medications.[9,30,41] For use in primary health care practice, a simpler tool, such as a questionnaire that could identify patients with core symptoms of NP would be valuable.

The painDETECT Questionnaire (PDQ) was developed and validated to support the identification of NP components in patients suffering from chronic pain of different origin.[12] It has previously been shown that pain descriptors of the PDQ correlate with QST items testing–related pain thresholds in patients suffering from radiculopathy, but not in patients suffering from fibromyalgia.[36] We undertook this exploratory analysis in a larger sample prospective study because the PDQ was developed before a clear definition of NP was established.[37] Therefore, it was never part of the validation process of PDQ to investigate whether different types of sensory loss (as described above) present with different PDQ profiles or if single PDQ items are sensitive to types of sensory loss in a clinical examination. This would be useful to validate the PDQ not only as a screening tool for NP itself but also for different sensory subtypes of NP. The aim of the study was to analyze whether the overall PDQ score or its items reflect phenotypes of sensory loss in NP as determined by QST.

Within the European consortia Innovative Medicines Initiative Europain and Neuropain, both QST data and PDQ results of patients with NP assessed by pain research units across Europe were gathered in a central database, enabling an analysis of the correlation between QST obtained from somatosensory profiles of thermal and/or mechanical loss of function and PDQ profiles.

2. Methods

2.1. Patient cohort

The joint European database of the Innovative Medicines Initiative Europain and Neuropain consortia, 2 prospectively collected cohorts using identical study protocols conducted in parallel, contains 580 data records of patients with lesions or diseases of the somatosensory nervous system. The recruitment of these 2 prospective studies started in March 2011 and the database was merged and finally closed in December 2013. Both studies were approved by local ethics committees according to local regulations. All subjects provided signed written informed consent according to the current version of the Declaration of Helsinki for participation in the respective study and transfer of the study records into the central database. Only patients with complete QST and PDQ (unless in the case of a painless neuropathy) were included into the database. All centers and investigators underwent a strict quality assessment and certification process to ensure that we were able to pool future data across sites and countries.[40,28] A confirmatory analysis of heterogeneity between the participating centers with healthy subjects and patients with painful PNP or peripheral nerve injury showed a high degree of homogeneity among the different centers, making it possible to analyze the database as a homogenous group.[39] With only few exceptions, patients performed QST and PDQ on the same day. In case of deviation from the same day assessment, the PDQ was completed within a week after QST. Both QST and PDQ are reported to be highly reliable to normal test–retest fluctuation within a few days.[14,22]

The main inclusion criterion for this analysis was that all patients in the central database had pain for more than 6 months, with a mean pain intensity in the last 4 weeks of ≥2 on a Numerical Rating Scale, 0 to 10 and pain due to PNP, peripheral nerve injury, postherpetic neuralgia, radiculopathy, or trigeminal neuropathy (n = 336). As we were aiming to specifically explore commonalities across NP entities and to obtain as generalizable information as possible, diagnoses were pooled and not analyzed separately.

2.2. Europain consortium

The EUROPAIN project (http://www.imieuropain.org) was founded in 2009 and aims to improve the treatment of patients with long-term pain and consists of academic study groups working on pain research from Germany, Denmark, and the United Kingdom. A Spanish small- and medium-sized enterprises and Europe's most active pharmaceutical companies working on pain also contributed. Data for this study were collected by the following centers: Ruhr-University Bochum, Germany, University of Schleswig Holstein, Kiel, Germany, Technische Universität München, Munich, Germany, and Aarhus University, Denmark. The ethics committee of each center approved the study protocol separately.

2.3. Neuropain consortium

The NEUROPAIN project is an investigator-initiated study consisting of several researchers in the field of NP research within Europe (principal investigator: R.B.) and aims to characterize subgroups of patients with NP. Data for this study were collected by the following centers: Ruhr-University Bochum, Germany, University of Schleswig Holstein, Kiel, Germany, Technische Universität München, Germany, Aarhus University, Denmark, Université Versailles-Saint-Quentin, Versailles, France, Helsinki University Central Hospital, Finland, Karolinska Institutet, Stockholm, Sweden, Benedictus Hospital Tutzing, Germany, Imperial College, London, United Kingdom, Heidelberg University, Germany, and Neuroscience Technologies, Ltd, Barcelona, Spain. The ethics committee of each center approved the study protocol individually.

2.4. Central database

Each study center used the computer-assisted program Neuroquast (Statconsult, Magdeburg, Germany) for data entry locally. Study records were implemented into the central database on a monthly basis.

2.5. Investigations

Standardized patient assessments were performed by all the pain centers using the same case report forms.

2.6. Inclusion/exclusion criteria

For inclusion of patients in the central database, the diagnosis of underlying etiology and classification of pain as neuropathic were made by an experienced physician with a qualification in pain medicine and documented in the local center. Inclusion criteria for each diagnosis were as follows:

(1) PNP: pathological electroneurography or pathologically decreased VDTs at 2 of 4 sites (<5/8) at the lower limb,[18,31]

which could not be explained by another disease, or pain with PNP-type location and evidence of small fiber neuropathy based on skin punch biopsy, laser-evoked potentials, or bedside thermal testing, which could not be explained by another disease.
(2) Peripheral nerve injury: history of traumatic nerve injury of the distal upper or lower limb and sensory-motor abnormalities confined to the innervation territory of the injured nervous structure.
(3) Postherpetic neuralgia: unilateral zoster rash in the facial or thoracic area with post-zoster scarring, hypopigmentation or hyperpigmentation in the affected dermatome or sensory deficit in the area of the previous zoster rash determined by bedside testing.
(4) Radiculopathy: pain in the L5 and/or S1 dermatome and positive straight leg raising test or sensory deficit within the matching dermatome or diminished Achilles tendon reflex for S1 lesions and magnetic resonance imaging of the lumbar spine confirming nerve root impairment by a herniated intervertebral disk or electromyography showing denervation in the L5 or S1 territory.
(5) Trigeminal neuropathy: idiopathic sensory trigeminal neuropathy or iatrogenic mandibular neuropathy (ie, inferior alveolar or lingual nerve neuropathy after various kinds of intraoral procedures) or trigeminal neuropathy secondary to compression, trigeminal neuropathy secondary to percutaneous lesions of the Gasserian ganglion, and sensory loss in the neuroanatomical adequate trigeminal territory.

Exclusion criteria for entry in the database were age <18 years, missing informed consent, insufficient language skills or other communication problems, pain treatment by topical local anesthetics for ≥7 days in the last 4 months or by topical capsaicin in the last 6 months, comorbidities treated by anticonvulsants or antidepressants, other pain locations with pain intensities ≥6 on ≥15 days per month, other severe systemic or focal diseases of the central nervous system (eg, stroke, spinal cord lesion), spinal canal stenosis, peripheral vascular disease (Fontaine stage II or higher), pending litigation, and major cognitive or psychiatric disorders. In the cases of unilateral NP syndromes, patients with contralateral neuropathy or painful conditions of the contralateral limb were excluded. Data sets were excluded in the case of incomplete records (eg, no precise diagnosis available, more than 2 missing variables of the QST in the affected area, no information about age, gender, or other demographic data).

2.7. painDETECT Questionnaire

The PDQ is a validated screening tool developed to aid in identifying NP and NP components.[12,6] It comprises 9 items regarding the severity, course, quality, and nature of the patient's pain. It screens for symptoms like burning, tingling or prickling sensations, pain evoked by light touch, thermal stimuli or light pressure, spontaneous pain attacks, and numbness, which are linked to neuropathic components of the pain. Patients can rate the intensity of symptoms on a 6-point Likert scale from "never" to "very strongly." For the present analysis, the 7 questions for the grading of sensory symptoms were used.[12]

2.8. Quantitative sensory testing

In accordance with the German Research Network on Neuropathic Pain (DFNS) protocol, QST comprises a standardized battery of 13 different thermal and mechanical parameters and assesses both afferent small fiber (unmyelinated and thinly myelinated C and Aδ fibers, respectively) and large fiber functions of the thickly myelinated Aβ fibers.[34,23] The following parameters are part of the protocol: CDT and WDT, alternating warm and cold stimuli (thermal sensory limen) during which the number of paradoxical heat sensations is counted, cold and heat pain thresholds (HPT), mechanical detection threshold (MDT), VDT, mechanical pain threshold for pinprick, pressure pain threshold for blunt pressure, mechanical (pinprick) pain sensitivity, dynamic mechanical allodynia (DMA) for brush, cotton wool and Q-tips, and pain sensation to repetitive pinprick stimuli in so-called wind-up ratio.

2.9. Z-transformation of quantitative sensory testing data

After z transformation, QST values can be directly compared between patients, across different testing areas, age decades, and gender. All QST values were z-transformed separately for each parameter (with the exception of paradoxical heat sensations and DMA).[28,33,34] Z values above 1.96 indicate an individual level abnormal gain of function where the patient is significantly more sensitive to the tested stimuli compared with healthy controls of the same gender, of comparable age, and tested in the same area (hyperesthesia, hyperalgesia). Z scores below −1.96 indicate an individual level abnormal loss of function referring to a significantly lower sensitivity of the patient (hypoesthesia, hypoalgesia). A z value of 0 indicates the mean of the healthy control group matched for age, gender, and testing site; all values above 0 indicate gain of function, and all values below 0 indicate loss of function. This procedure leads to sensory profiles of groups of patients that can be graphically displayed on one common scale for sensory loss and gain.

2.10. Subgrouping of patients

Based on their QST profile, all data sets were subgrouped according to the type of sensory loss, partial or total:
(1) Patients with no abnormal loss of sensation in thermal and mechanical detection parameters
(2) Patients with isolated abnormal loss of thermal sensation (loss of detection in the CDT and/or WDT, representing pure loss of small fiber function)
(3) Patients with isolated abnormal loss of mechanical sensation (loss of detection in MDT and/or VDT, representing pure loss of large fiber function)
(4) Patients with both abnormal loss of thermal and mechanical sensation (representing loss of small and large fiber function).

2.11. Statistics

The IBM Statistical Package for the Social Sciences (SPSS) version 22 was used for all statistical analyses. We analyzed each PDQ item for significant fiber effects in a 2-factor analysis of variance, where each factor represents either the function of small or large fibers. Factor 1 was an abnormal loss of thermal sensation (subgroups 1 and 4, loss of small fiber function), and factor 2 was an abnormal loss of mechanical sensation (3 and 4, loss of large fiber function).

3. Results

3.1. Characteristics of patients

Neuropathic pain in this combined patient sample from 3 consortia was most frequently caused by PNP (49%), peripheral

nerve injury (24%), and radiculopathy (18%) (**Table 1**). Across the cohort and the different etiologies, gender was almost equally distributed and the average age was 58 (±15) years.

3.2. Quantitative sensory testing subgrouping

According to the cutoff of *z* values below −1.96 (95% confidence interval of values found in a healthy control group matched in age and gender, assessed in the same area), 79 patients had no sensory loss (**Fig. 1A**), 55 patients had only thermal sensory loss (**Fig. 1B**, CDT and WDT), 69 had only mechanical sensory loss (**Fig. 1C**, MDT and VDT), and 133 a combination of the 2 (**Fig. 1D**). Mean *z* scores in the groups with significant sensory loss according to the cutoff were lower than −1.96. In 86 cases (46%), if a patient had loss in either CDT or WDT, the same was true for the other quality. For MDTs, this was similar, although slightly less pronounced (78 cases [39%]). Patients without individually diagnosed sensory loss nonetheless had slightly negative mean *z* scores (around −1) for detection thresholds and slightly positive mean *z* scores for pain measures; this group also had the most pronounced pinprick hyperalgesia and DMA. Patients with loss of thermal sensation (subgroups 2 and 4) also had a loss for heat pain sensitivity (HPT, mean value for loss of thermal sensation = −0.98, $P < 0.001$, mean HPT value for loss of both thermal and mechanical sensation = −1.25, $P < 0.001$). Patients with loss of mechanical sensation (subgroups 3 and 4) also had minor loss of thermal sensation with mean *z* values approximately −1.00 in CDT and WDT.

Pain intensity did not differ between groups of sensory loss, neither current nor mean or maximum pain of the last 4 weeks. There were no significant differences in the frequency of intake of medication (**Table 2**).

3.3. Effects of loss of sensation quantitative sensory testing phenotype on painDETECT Questionnaire items

The values for the overall PDQ score (**Fig. 2A**) are similar among the 4 subgroups. Consequently, there are no significant effects in the analysis of variance (**Table 3**).

Panels B to D reflect PDQ items referring to pain evoked by external stimulation. "Pain evoked by light touch" (**Fig. 2B**) is reported significantly less frequently in patients with abnormal loss of mechanical sensitivity and significantly more frequently in patients with abnormal loss of thermal detection. Thus, this PDQ item seems to require intact large fiber innervation and to be facilitated by impaired small fiber function. The patient groups did not differ significantly with respect to their PDQ report of pain evoked by slight pressure, although these reports were on average 0.3 scores less, when QST had demonstrated loss of mechanical sensitivity.

Within the items regarding spontaneous sensations (**Fig. 2E–H**), no differences could be found between patients with normal thermal sensitivity and loss of thermal perception. Burning sensations (**Fig. 2E**) were reported significantly less frequently by patients with loss of mechanical sensation in comparison with patients with normal MDTs, and numbness was reported significantly more frequently in patients with impaired large fiber function (**Fig. 2H**).

4. Discussion

Although loss of nerve fiber function is an important sign of neuropathy, sensory loss is not represented well in the existing screening tools, possibly because they were created before the new definition of NP emphasized the importance of the clinical sensory examination.[37] The present analysis reveals that the overall PDQ score, the primary outcome measure, is not related to the presence or absence of sensory loss as determined by QST. However, 4 individual PDQ items were partly sensitive enough to reflect loss of small or large fiber function: numbness, burning sensations, pain evoked by light touch, and pain evoked by cold or heat. Whereas numbness and burning sensation reflect perceptions without external stimulation, the other 2 items may be considered to reflect self-examination by the patient.[5]

4.1. Subjective report of numbness

In our data, "numbness" is more often reported by patients with loss of mechanical sensation than by those with intact mechanical perception. This is consistent with the interpretation that the subjective feeling of numbness is related to loss of large fiber or dorsal column functions, for example, in a case of unilateral damage to the spinal cord,[13] where numbness was reported for the side with tactile loss and not the contralateral side with thermal loss. Although our data support this concept, the association was not strong enough to allow identification of patients with large fiber loss using this PDQ item.

4.2. Subjective report of burning

Reports of "burning sensations" were most frequent in patients with intact mechanical and thermal sensitivity according to QST. This is also the only group where heat pain testing revealed heat hyperalgesia in QST (**Fig. 1A**), whereas thermal

Table 1

Characteristics of patients and QST results.

Disease	Polyneuropathy	Peripheral nerve injury	Radiculopathy	Postherpetic neuralgia	Trigeminal neuralgia	Total
Demographics						
No. of patients	164 (49)	79 (24)	61 (18)	23 (7)	9 (3)	336 (100)
Female	88 (54)	45 (57)	24 (39)	7 (30)	1 (11)	165 (49)
Age, mean ± SD, y	62 ± 15	47 ± 11	59 ± 12	69 ± 12	54 ± 18	58 ± 15
Subgroups (based on QST results)						
No loss of sensation	32 (20)	20 (25)	18 (30)	7 (30)	2 (22)	79 (24)
Loss of thermal sensation	24 (15)	14 (18)	13 (21)	4 (17)	0 (0)	55 (16)
Loss of mechanical sensation	38 (23)	12 (15)	14 (23)	2 (9)	3 (33)	69 (21)
Loss of mechanical and thermal sensation	70 (43)	33 (42)	16 (26)	10 (43)	4 (44)	133 (40)

Values are given as n (%).
QST, quantitative sensory testing.

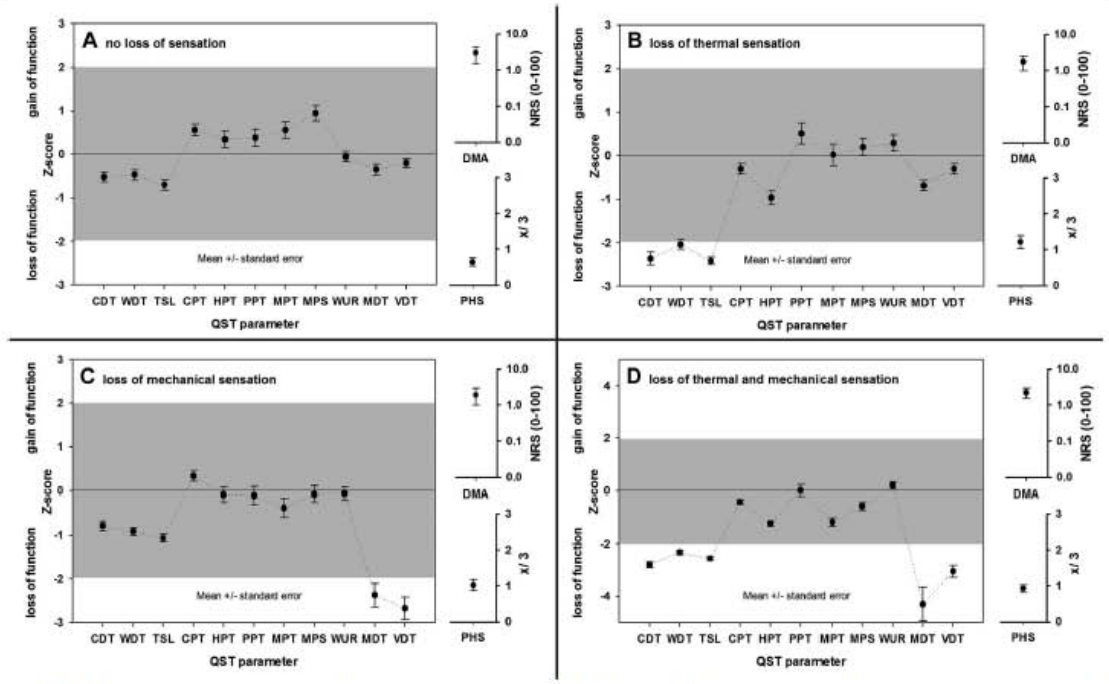

Figure 1. QST–*z*-profile separately for patients with no loss of sensation (A), loss of thermal sensation (B), loss of mechanical sensation (C), and loss of thermal and mechanical sensation (D). Values are presented as mean ± SEM. Values between −1.96 and +1.96 represent the 95% confidence interval in an age-matched and sex-matched healthy control population, tested at the same body region. Values below 0 indicate a loss of function (eg, increased detection thresholds), whereas values above 0 indicate gain of function (eg, decreased pain thresholds). CDT, cold detection threshold; CPT, cold pain threshold; DMA, dynamic mechanical allodynia; HPT, heat pain threshold; MDT, mechanical detection threshold; MPS, mechanical pain sensitivity; MPT, mechanical pain threshold; NRS, Numerical Rating Scale; PHS, paradoxical heat sensations; PPT, pressure pain threshold; QST, quantitative sensory testing; TSL, thermal sensory limen; VDT, vibration detection threshold; WDT, warm detection threshold; WUR, wind-up ratio.

sensory loss was associated with hypoalgesia to heat (**Fig. 1B and D**). These observations are consistent with the concept that discharges from "irritable nociceptors" may lead to burning sensations.[11,9] In previous studies, burning pain (and burning sensation) has been discussed as being associated with loss of thermal sensation.[26] Unexpectedly, there was no difference between patients without sensory loss and patients with isolated loss of thermal sensation on the item burning sensations. Based on the thermal grill illusion and on observations in patients with multiple sclerosis, it had been suggested that burning pain might be due to a disinhibition of a heat-sensitive neural pathway that is normally suppressed by a cold-sensitive pathway.[7,16] Our observations are at variance with this concept of a disinhibition at the thalamocortical level.

Table 2

Pain intensity and medication in relation to sensory loss.

	Normal sensation	Loss of thermal sensation	Loss of mechanical sensation	Loss of thermal + mechanical sensation	P*
Pain intensity					
Current	4.8 ± 2.2	5.2 ± 2.3	4.6 ± 2.0	5.3 ± 2.2	0.126
Maximum (4 wk)	7.7 ± 1.8	7.9 ± 1.8	7.7 ± 1.6	7.8 ± 2.0	0.943
Mean (4 wk)	5.8 ± 1.9	6.1 ± 1.8	5.6 ± 1.8	6.2 ± 2.0	0.131
Current medication, n (%)					
NSAID	7 (9)	13 (24)	11 (16)	30 (23)	0.058
SNRI	9 (12)	6 (11)	10 (14)	13 (10)	0.798
SSRI	3 (4)	5 (9)	7 (10)	21 (16)	0.055
Anticonvulsants	26 (33)	19 (35)	16 (23)	45 (34)	0.408
Tricyclic	18 (23)	15 (27)	22 (32)	52 (39)	0.089
Opioid	13 (17)	13 (24)	13 (19)	41 (31)	0.081
Other	5 (6)	4 (7)	9 (13)	17 (13)	0.359

Mean, maximum, and current pain intensity are presented as mean ± SD (0–10 numerical rating scale). For medication, multiple answers per patient were possible.
* Result of a 2-way analysis of variance (pain) or χ^2 test (medication).
NSAID, nonsteroidal anti-inflammatory drug; SNRI, serotonin-norepinephrine reuptake inhibitors; SSRI, selective serotonin reuptake inhibitors.

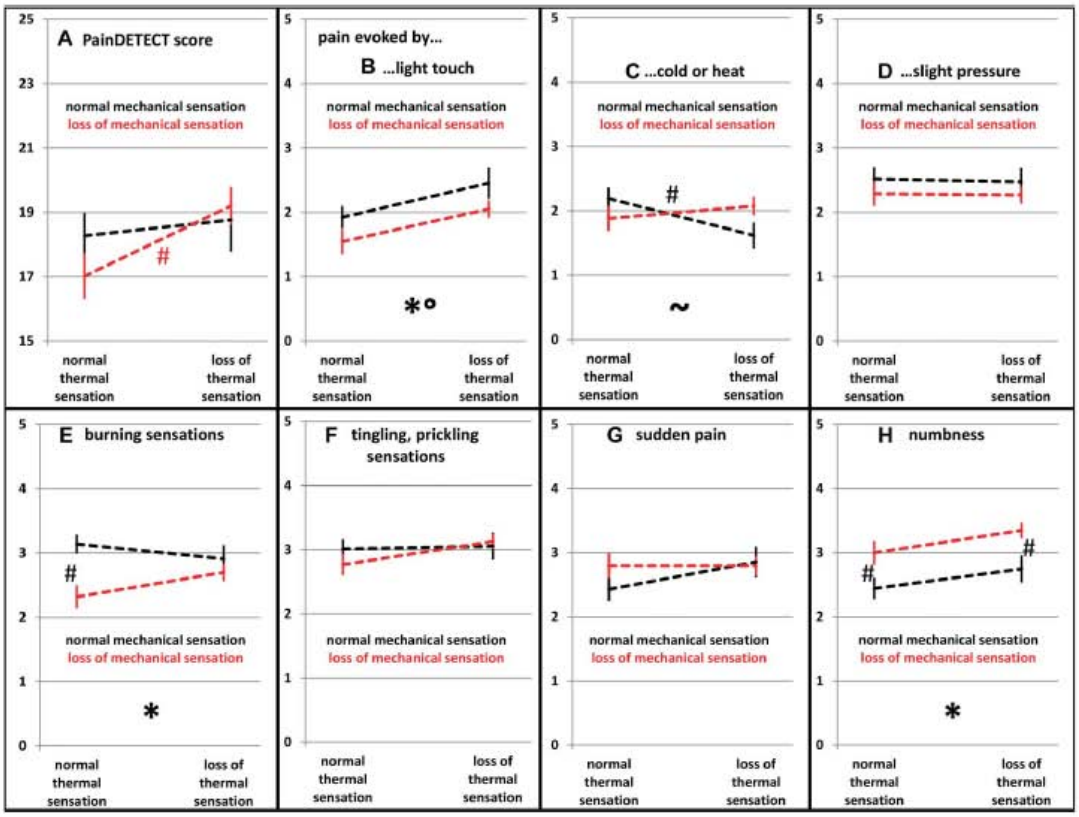

Figure 2. Interaction plot for effects of small and large fiber deficits on total PDQ score (A) and single PDQ item scores of evoked pain (B–D) and spontaneous sensations (E–H), presented as mean ± SEM. For each item, the values on the left end of each line indicate normal small fiber function (no loss thermal sensation), and values on the right indicate impaired small fiber function (loss of thermal sensation). Black lines indicate normal large fiber function (no loss of mechanical sensation), and red lines indicate impaired large fiber function (loss of mechanical sensation). *Significant large fiber effect, °significant small fiber effect, ~significant interaction effect in the analysis of variance ($P < 0.05$; Table 3). #Significant post hoc test between patient groups with normal vs loss of mechanical sensation (#on the left or right end of the graph) or between patient groups with normal vs loss of thermal sensation (#in the middle of the graph). PDQ, painDETECT Questionnaire.

4.3. Self-report of pain evoked by light touch

The mean report of "pain evoked by light touch" is decreased in patients with mechanical sensory loss and increased in patients with loss of thermal sensation in QST. Pain evoked by light touch is associated with static or DMA, which has been described in patients with loss of thermal sensation.[17,19,24]

4.4. Sensory phenotype, medication, and pain intensity

Pain intensities did not differ significantly among the groups of sensory phenotypes. There are 2 reasons likely underlying this finding: (1) QST is not able to assess pain intensity itself, and loss of sensation is not correlated with pain intensity; (2) QST and parts of the PDQ describe evoked types of pain, whereas Numerical Rating Scale and the rest of the PDQ capture the ongoing pain. Medication also did not differ among the sensory phenotype groups, although there was a tendency for the use of nonsteroidal anti-inflammatory drugs and opioids to be associated with loss of thermal sensation and the use of tricyclic antidepressants and selective serotonin reuptake inhibitors (SSRIs) to be associated with the large loss of mechanical sensation (all $P < 0.1$). These patterns may influence the results obtained, as pharmacological treatment has the potential to influence pain qualities and sensory descriptors.[30] The broad spectrum of analgesics taken reflects the situation in which the patients were recruited: when referred from primary care, where their pain could not be managed.

4.5. Outlook and conclusions

The NP results from heterogeneous etiological, genetic, and environmental causes, possibly leading to different profiles in sensory testing and questionnaire data.[2,3,25,38] This, in turn, leads to difficulties in predicting the outcome of currently available therapies.[2] Several recently conducted proof-of-concept-controlled trials for promising drugs in clinical development have failed to demonstrate an effect superior to placebo.[10,21] The reason for this may be that these drugs are aimed at specific targets that may only be relevant in a subgroup of patients with NP, leading to the inclusion of a large group of patients who are unlikely to respond to a drug targeting a pathomechanism that is

Table 3

Results from the 2-way analysis of variance analyzing the effects of impaired small or large fiber function (abnormal loss of thermal or mechanical sensation) for the PDQ score and each PDQ item presented as F value (*P* value).

	Model	Small fiber effect	Large fiber effect	Small × large interaction
PDQ score	1.69 (0.168)	3.08 (0.080)	0.29 (0.593)	1.24 (0.267)
Burning sensation	**4.24 (0.006)**	0.19 (0.665)	**8.85 (0.003)**	3.11 (0.079)
Prickling sensation	0.90 (0.440)	1.60 (0.207)	0.29 (0.590)	1.00 (0.318)
Sudden pain	0.73 (0.536)	1.18 (0.278)	0.63 (0.428)	1.18 (0.277)
Numbness	**6.98 (0.000)**	3.64 (0.057)	**11.59 (0.001)**	0.02 (0.898)
Light touch	**3.16 (0.025)**	**7.82 (0.005)**	**4.43 (0.036)**	0.00 (0.953)
Cold or heat	1.62 (0.184)	1.04 (0.308)	0.16 (0.687)	**4.25 (0.040)**
Slight pressure	0.64 (0.589)	0.03 (0.858)	1.39 (0.239)	0.01 (0.941)

Values significant on $P < 0.05$ are given in bold.
PDQ, painDETECT Questionnaire.

not underlying their pain condition.[8,9,30] Because pathophysiological mechanisms cannot readily be identified in patients, surrogate measures have been suggested that are likely to reflect the underlying pathophysiological mechanisms, for example, a classification strategy based on the functional gain or loss of mechanical and thermal sensations assessed by QST was introduced.[29] Furthermore, PDQ has been used for subgrouping of patients according to their pattern of sensory abnormalities. These studies used a hierarchical cluster analysis for segmentation and identified 5 subgroups of patients with diabetic painful neuropathy, postherpetic neuralgia, and painful radiculopathy according to their sensory symptoms.[3,4] Therefore, PDQ may provide an easy and beneficial way of distinguishing patients according to their sensory profile. Patients could benefit from an early identification of NP and sensory dysfunction phenotype and could avoid insufficient treatment. This approach could also help in deciding whether further investigations are necessary, which may also reduce costs for the health care system.

Questionnaires like the PDQ are easy-to-use tools in daily clinical practice for gathering information on subjective reports and self-examination,[5] whereas bedside sensory testing and QST are validated methods to evaluate the sensory profile in selected pain areas in patients with NP. In contrast to, for example, conventional nerve conduction studies, both bedside sensory testing and QST can assess the function of thin myelinated and unmyelinated (small) nerve fibers.[27,34] Quantitative sensory testing and bedside sensory testing address the diagnostic dilemma, that is, that patients suffering from isolated loss of small fiber function cannot be identified by a standard neurological examination[17] or nerve conduction studies, which cannot distinguish between healthy subjects and patients suffering from neuropathies with isolated loss of small fiber function.[20,32] In addition, QST is suitable for assessing pain thresholds, detection thresholds to nonpainful stimuli, and evoked pain, but it is unable to capture spontaneous pain, as are conventional electroneurographic techniques. Other diagnostic methods like skin biopsy or corneal confocal microscopy reveal information about the structure of small nerve fibers only.[17,19,26]

The development of specialized questionnaires for the detection of NP phenotypes may improve clinical practice in the treatment of NP outside specialized centers. A simple tool would be beneficial in assisting the primary care physician to select patients needing referral to a specialist for further testing. At present, such screening tools are not sensitive enough to document sensory loss, which is an important criterion in diagnosing NP.[37] It is important to note that QST or skin biopsy cannot be replaced but only supported by such screening tools or rather a suggestion can be made, if a skin biopsy or QST would be useful. An additional improvement could be a validated and reliable battery of bedside testing,[35] which could be compared in terms of sensitivity and specificity with both pain questionnaires and QST. Such an approach would go far towards working out the value of all 3 methods in relation to each other.

In conclusion, these results demonstrate that the PDQ score is not sensitive enough to distinguish different types of sensory loss in patients with NP. Although our results indicate that there are differences in the responses to 4 items between *groups* of patients with one or the other kind of QST-detected loss of sensation, they do not provide clear information on an *individual* patient basis, as the mean differences are comparably small (approximately 1 point on a 0-5 scale) and show a huge overlap. painDETECT Questionnaire is unable to clearly separate between patients with varying types of loss of sensation, because its questions for loss of function are limited to numbness (and are completely missing in other pain questionnaires), whereas loss of function can be detected for all sensory qualities in QST. A more complex analysis, identifying items that differentiate between types of sensory loss in a new battery of questions would be an interesting approach for a future study. Some domains may give more detailed information if they are divided into 2 items, eg, the item *cold or heat pain*, one for pain evoked by cold and one for pain evoked by heat. Separate items for the different forms of hyperalgesia or allodynia might also be of value. In addition, questions about autonomic disorders, which commonly appear in patients with small fiber neuropathy,[24] would be helpful. Pursuing this next step in the development of a convenient screening tool could help identify different phenotypes involved in NP.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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CLINICAL REPORT

Complex Regional Pain Syndrome in Children: a Multidisciplinary Approach and Invasive Techniques for the Management of Nonresponders

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■ **Abstract:** Complex regional pain syndrome (CRPS) is multifactorial condition with complex pathogenesis characterized by spontaneous or stimulus-induced pain that is disproportionate to the inciting event. It is also commonly accompanied by a myriad of autonomic and motor disturbances in highly variable combinations. This condition has been underreported in children until recently. Consequently, the management of CRPS in the pediatric population presents an even greater challenge than in adults, partly because there is a lack of clinical data concerning the efficacy of the diverse treatment methods available, and partly because successful treatment of CRPS involves a multidisciplinary approach. In this retrospective case series, a multidisciplinary management plan is presented in 10 children for whom the standard noninvasive treatment was unsuccessful. Within this management plan, novel drugs were included such as the capsaicin 8% patch, in addition to invasive techniques in patients who did not respond to noninvasive therapies. ■

Key Words: CRPS, complex regional pain syndrome, pediatric pain, neuropathic pain, epidural analgesia, infusion pumps, implantable, capsaicin, multidisciplinary pain management, clinical case series

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INTRODUCTION

Complex regional pain syndrome (CRPS) is a term refined by the International Association for the Study of Pain (IASP) to describe disorders characterized by spontaneous or stimulus-induced pain that is disproportionate to the inciting event.[1–3] The disease often includes a wide variety of autonomic and motor disturbances in highly variable combinations[4,5] in addition to a mixture of noxious sensations (positive symptoms) and sensory loss (negative symptoms).[3,6,7]

CRPS has been extensively studied in adults, but studies in children are scarce. Previously, it was doubtful that this condition even existed in children; however, numerous recent articles have reported CRPS in children.[8,9] Furthermore, several authors have highlighted differences in the pediatric presentation compared to that of adult CRPS.[10] Approximately 90% of reported cases are girls between 8 and 16 years of age, with the lower limbs most commonly affected. Leading symptoms are intensely burning pain, along with cold and mechanical allodynia, dysesthesia, and paresthesia. Additionally, signs of autonomic dysfunction, movement problems and psychological difficulties are also regularly present.[8,9,11–13]

Not only does diagnosing CRPS pose a significant challenge, but the timing of the diagnosis can also determine the prognosis.[8,11,14,15] Furthermore, prompt and accurate management is vital, as the cornerstone of therapy is to restore function of the affected limb. Recognized therapies include a combination of pharmacotherapy, physical therapies, and psychotherapy where appropriate.[1,14,16–18] There is evidence that the pediatric population responds better to noninvasive approaches.[19] As a result, this style of management is growing across Europe and the United States.[9,17] Nonetheless, an exact treatment model or algorithm has not yet been established. Unfortunately, not all patients respond successfully to conservative management, making further interventions a necessity. Many patients who fail to progress with physical therapy may require additional or more aggressive pain therapy, such as spinal cord stimulation (SCS) or intraspinal analgesic infusion.[13,20,21] The significance of invasive therapies in children who do not respond to conventional treatments or medications has not been established, although most therapies used in adult CRPS have been tested in children, including spinal stimulation or drug infusion, TENS, and sympathetic blockade under general anesthesia. One can find numerous reports in the literature demonstrating success using these procedures,[22–26] providing doctors with further alternatives when the noninvasive options are not enough. Consequently, we hypothesized that children who do not respond adequately to conservative measures may have the same opportunity to reduce their symptoms with invasive treatments.

This article reports on the course and management of 10 children diagnosed with CRPS who did not respond successfully to conservative pain management therapies presenting to our pain clinic.

METHODS

This is a clinical series reporting on 10 cases of children (5 males and 5 females) between 8 and 13 years old who presented to *the Pain Treatment Unit of the Hospital Regional Universitario de Malaga* between July 2010 and May 2014. These patients had been diagnosed with CRPS, but previous pain therapy was not successful. Approval from the hospital's institutional review board was obtained before the study was conducted. For this study, all patients with CRPS were recruited and diagnosed at the pain unit. Next, we followed a treatment pathway using conventional management first and invasive treatments if the patients did not respond, described in detail below. Follow-up appointments were carried every 14 to 20 days until resolution of the symptoms or after a year from the diagnosis at the pain unit.

All patients between 5 and 16 years old who were evaluated for long-lasting limb pain and possible CRPS in our Pain Treatment Unit from the May 2010 to May 2014 were included in a process to determine whether they met the modified IASP Budapest criteria for the diagnosis of CRPS.[27] The final diagnosis was made in all cases by a senior pain medicine consultant using the clinical diagnostic criteria for CRPS proposed by the Budapest IASP consensus group; this was necessary for inclusion in the study. Children with neuropathic or limb pain who did not meet these criteria were excluded from the study. The 10 children meeting the Budapest criteria also matched the less rigorous current IASP diagnostic criteria for CRPS.

In this study, all included patients were referred to the Pain Treatment Unit because of limited or no response to noninvasive treatment (physical therapy, NSAIDs, and acetaminophen). All patients were referred to the Pain Treatment Unit by the services of Traumatology and Pediatric Rheumatology, with a median time for the referral of 18 weeks. The medical and clinical documentation of the patients who were referred to our pain unit and who accomplished the diagnosis of CRPS were reviewed since the presumptive diagnosis of CRPS commenced at the referring service. These patients were followed for at least 12 months after the diagnosis was made at the pain unit. The length of time taken to make the diagnosis was calculated from the onset of symptoms. Once diagnosis was made, the length of time to recovery was calculated. Full recovery was defined as recovery with no recurrence of symptoms within the initial 5 months following resolution. Recurrences after this time were considered new episodes.

Treatment Approach

In this study, the same treatment protocol was followed for every patient after exhaustive clinical investigations (Figure 1). After the initial consultation and diagnosis, the first option was always pharmacological treatment for neuropathic pain, together with physiotherapeutic management and psychological therapy where needed. The physiotherapy treatment prescribed was exercise to facilitate motion, strength, and proprioception among other qualities, together with sensory desensitization. Psychological treatment entailed daily individual and

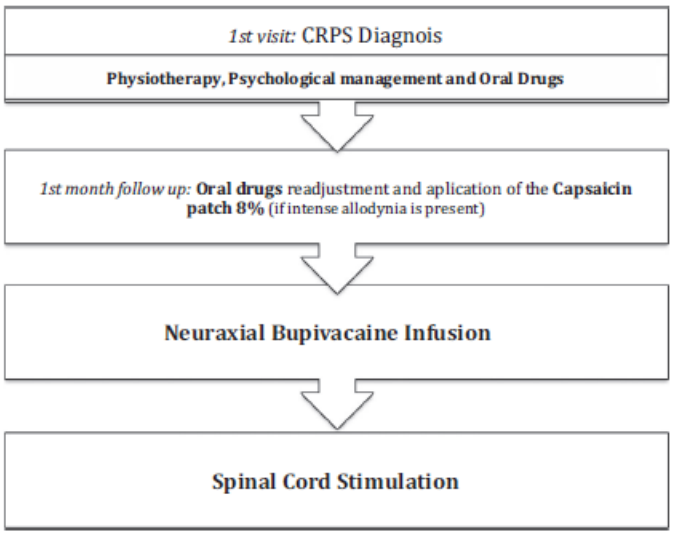

Figure 1. Treatment approach. At the first visit, CRPS diagnosed is made and a new treatment plan is recommended. This gathers oral medication as gabapentinoids, antidepressants, and analgesics. Weekly follow-ups allow us to readjust the medication and add topical capsaicin if allodynia is intense. 40 to 50 days after conservative treatment has been started, if this has not been effective neuraxial bupivacaine is tested over 2 weeks (Figure 1). If neuraxial analgesics are not effective either, SCS is tested and finally implanted if the treatment is successful. Epidural local analgesic infusion. All patients who were going to receive this treatment were hospitalized the day before. The epidural catheter was inserted via the L3–L4 interspace and advanced into the epidural space using an 18G Thuoy needle under fluoroscopy guidance. This technique was performed under general anesthesia and with the patient in *lateral decubitus* position. The catheter was then tunneled subcutaneously and connected to the pump. Pump–catheter system integrity was verified postoperatively. Treatment was started after the patient got recovered from the anesthesia, bupivacaine 0.25% at 2 mL/hour. This dose was titrated until reach the 4 mL/hour over 72 hours. All patients continued at the hospital for 24 hours after the intervention prior discharge. This treatment continued for 14 days. After this time, the epidural catheter got carefully removed.

group-based cognitive behavioral therapy (CBT). Pharmacological treatments included gabapentin, pregabalin, or antidepressants such as amitriptyline, prescribed alone or in combination with other analgesic drugs such as tramadol. Additionally, if the patient presented intense allodynia and/or severe hyperalgesia, the capsaicin 8% patch was offered as an option to support pharmacological treatment. For those children who did not respond to this type of treatment after a period of 3 to 5 weeks, a more invasive treatment was chosen. Treatment responsiveness was determined by a decrease of at least 33% on the visual analog scale (VAS) and functional improvement determined independently by the patient, the physiotherapist, and one of the consultants at the pain unit. At this point, the first step was neuraxial analgesia. Under general anesthesia, we placed a lumbar epidural catheter for bupivacaine infusion of 2 weeks (Figure 1). After this time, the catheter was removed and the subject was evaluated. The VAS and motor dysfunction were assessed and compared with previous records, and possible side effects were also recorded. If pain persisted, our next step was to surgically place a spinal cord stimulator under general anesthesia. Correct positioning of the electrode and stimulation parameters were set once the patient was awake and fully recovered from the anesthesia. A trial stimulation with a temporary percutaneous extension was performed for about 2 weeks before permanent implantation of the pulse generator.

Outcome measures included spontaneous and evoked pain (VAS), the presence of dysesthesia, allodynia, hyperalgesia, sensitivity to cold, dysautonomic signs, motor dysfunction (Functional Disability Inventory [FDI]),[28] ability to weight-bear, analgesic consumption, and school attendance. All of these items were assessed at periodic appointments every 2 weeks except for motor dysfunction, measured at the first

visit, at month 6, and at the end of the follow-up, that is, month 12. FDI classifies the physical disability as minimal, moderate, or severe. The appointments were made between the diagnosis and the resolution of symptoms to follow the evolution closely, to modify the treatment if needed, and to detect any possible side effects.

Case Reports

Case 2. Female, 13 years old. Without any trauma or other reasonable cause, she started to have pain in both feet 16 weeks before she was referred to the pain unit. CRPS was diagnosed and we rapidly started extensive psychiatric treatment and physiotherapy *(VAS 8/10)*. At that time, she had developed contractures and was confined to a wheelchair *(FDI 41)*. Pharmacological treatment was started as well, using the capsaicin 8% patch to minimize allodynia. A few weeks later, she reported progressive relief of her pain and motor disturbances. At follow-up 12 weeks later, she had no pain or dysautonomy and was contracture free *(VAS 0/10, FDI 5)*. Neither mechanical nor dynamic allodynia were evoked after 2 weeks of the initial treatment with the capsaicin patch; this was maintained until CRPS resolution.

Case 5. Female, 8 years old. The patient was diagnosed with CRPS after suffering a minor trauma to her left foot while playing at school. CRPS was resolved successfully with pharmacological treatment in a few days. However, 4 weeks later, without any new trauma or other reasonable cause, she started to have pain on the contralateral limb. Edema, redness, dysesthesia, paresthesia, allodynia, and motor problems were also present at the time of the diagnosis. First-line therapies of the management plan were implemented; however, the reduction in symptoms was modest, and the VAS score decreased by only by two points, while the FDI score remained over 40 (severe). Therefore, we implanted an epidural catheter under general anesthesia for continuous epidural infusion of bupivacaine (Figure 1). After 2 weeks, the pain and dysautonomic and motor symptoms were significantly reduced. At this time, the infusion was stopped and the catheter was removed. She reported a reduction in spontaneous pain from *VAS 9/10 to 1/10*. She continued with oral medication and further physiotherapy, leading to the complete cessation of pain for 12 more months. She is still pain free in both feet 18 months later. Motor dysfunction is minimal, and both autonomic and sensory abnormalities disappeared altogether in both feet.

Case 7. Male, 9 years old. After a minor ankle sprain while playing soccer, the patient developed persistent pain *(VAS 7/10)*, severe allodynia, and dysautonomic signs in the affected foot and lower leg. With our first-line management therapies, including the 8% capsaicin patch, he reported a significant reduction in allodynia but insufficient relief of pain *(VAS 5-6/10)* and motor disability *(FDI severe dysfunction)*. Neuraxial analgesia with a 2-week infusion of bupivacaine successfully diminished his pain and allowed him to recover full mobility of the limb once again *(FDI 10)*. Reductions were achieved in the VAS score and functional disability, that is, from 7 and 9 for evoked pain down to 0 and from severe to minimal, respectively, with this treatment protocol. After the epidural catheter was removed and the infusion was stopped, the subject continued to be pain free until the last follow-up, 24 months later *(VAS 0/10, FDI 3)*.

Case 9. Female, 13 years old. CRPS in the lower right limb developed a few days after an ankle fracture. The patient did not respond adequately to physical and pharmacological treatment during the first 30 weeks. She presented a *pain VAS score of 8/10* and severe motor impairment. She was thus referred to our pain unit where an epidural bupivacaine infusion was used to treat her pain. The pump was stopped and the catheter was removed 2 weeks after, obtaining excellent results. The girl reported pain relief with a *VAS for evoked pain from 9/10* down to *2/10*. However, the symptoms reappeared after the infusion was stopped. Following this, an SCS trial device was implanted with excellent results for 2 weeks *(VAS 1/10)*, allowing the patient to resume school and physical therapy; the impact of this treatment allowed for the patient to minimize her motor impairment. Two weeks after that improvement, we disconnected the stimulator device and continued with the physical and psychological therapies. Four weeks later, we achieved complete remission of the pain and additional symptoms, so we decided to remove the percutaneous electrode. Twelve months later, the patient remains pain free and with minimal functional disabilities.

Case 10. Female, 8 years old. She suffered a minor trauma to her left foot 17 weeks before she was referred to the pain unit with severe pain *(VAS 10/10)*, allodynia,

dysesthesia, and dysautonomic symptoms. Before her referral, she was treated with transdermal opioids and gabapentin among other analgesics, with poor results. At the pain unit, pharmacological treatment including topical capsaicin patches relieved her allodynia significantly but only modestly reduced her pain by two points on the VAS. Continuous epidural infusion of bupivacaine caused a temporary suppression of her spontaneous pain, but it came back when the infusion was stopped. SCS was decided on and about 7 months after the start of the pain, and an octopolar paddle electrode was implanted. Three weeks after the stimulation started, the symptoms were minimal and the patient regained complete limb functionality, returned to school and also started taking part in extracurricular activities *(VAS 0/10, FDI 6)*.

RESULTS

Following the management pathway described above, the outcomes were *noteworthy*. This clinical series shows that 9 of 10 patients had suffered a minor trauma or ankle sprain months before they were referred to the clinic, and presented with high intensity, neuropathic-type pain, along with significant motor and autonomic disturbances since the onset of injury (Table 1). Strikingly, one patient suffered trauma to the limb contralateral to where the symptoms were reported. The treated children had significantly reduced pain and improvement in other symptoms such as allodynia, dysesthesia, hyperalgesia, sensitivity to cold, and dysautonomic signs. CRPS even disappeared in the majority of cases. Although the children were all instructed to assess their pain using a VAS, it was not feasible to achieve this on a regular basis. The patients in our study primarily remained at home and only came to the pain unit for appointments. The children failed to do regular evaluations at home, so data were usually collected only at appointment times. Pain scores (VAS) decreased substantially during the study. The mean VAS for ongoing pain at the first visit was 7.7 (SD 0.9) and for evoked pain was 9.1 (SD 0.93), while 12 months after the first appointment, the mean VAS for the whole group was 0.3 (SD 0.4).

Physical functional disability was also reduced. Our patients showed severe disability at their first visit *(mean FDI: 32.4, SD 7.3)*; however, at the end of the study, they presented minimal disability *(mean FDI: 4.8, SD 3.1)*. These data parallel school absences, which declined from 60% to 0% throughout the study.

Within this series of patients, CRPS disappeared with oral medication and physical management in one patient. Three other children alleviated their symptoms by adding the capsaicin 8% patch to their pharmacological and physical management. Capsaicin treatment diminished mechanical and dynamic allodynia in all nine patients in whom it was used. Allodynia that was originally stated as "extremely unpleasant" at the beginning of the treatment in seven of ten children either disappeared completely or was just mildly unpleasant at the last appointment for all patients.

A second group of children needed further efforts to treat CRPS. Noninvasive management was unsuccessful in alleviating pain in these patients after a few weeks of treatment. Three of these children reduced their pain with 2 weeks of epidural bupivacaine by continuous infusion. None of these patients experienced a recur-

Table 1. Demographics and Clinical Data of Ten Cases of Children with CRPS

Case	1	2	3	4	5	6	7	8	9	10
Age (years)	13	13	10	11	8	10	9	11	13	8
Gender	Male	Female	Male	Male	Female	Female	Male	Female	Female	Female
Area affected	RLL	Bilateral	RLL	LLL	RLL	RLL	LLL	LLL	RLL	LLL
Trauma	Mild	No	Mild	Mild	Minor*	Mild	Minor	Mild	Mild	Minor
Time diagnosis-referral (weeks)	28	16	19	17	4	150	95	36	30	17
Ongoing pain (VAS)	6	8	7	8	8	7	7	9	8	9
Evoked pain (VAS)	9	10	8	9	10	7	9	10	9	10
Allodynia	+	++	++	+++	+++	++	+	+++	++	++
Motor dysfunction (FDI)	Minimal –moderate	Severe	Minimal	Moderate	Severe	Moderate –severe	Severe	Moderate –severe	Severe	Severe
Dysautonomic symptoms	+	+	+	++	++	++	+	++	+++	+
Other sensory disturbances	+	+	+	+++	++	++	++	+	+++	+

RLL, right lower limb; LLL, left lower limb; +, minor; ++, moderate; +++, severe.
*Trauma at contralateral limb where pain secondary appeared.

Table 2. Therapies Prescribed and Clinical Evaluations After a 12-month Follow-up

Case	1	2	3	4	5	6	7	8	9	10
Time diagnosis – referral (weeks)	28	16	19	17	4	150	95	36	30	17
Physical therapy	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Psychological therapy	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Pharacological Th.	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Capsaicin 8% patch	–	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Neuraxial bupivacaine infusion	–	–	–	–	Yes	Yes	Yes	Yes	Yes	Yes
SCS	–	–	–	–	–	–	–	Yes	Yes	Yes
Follow-up 12 months after the first visit at the pain unit										
Time referral – "CRPS free" (weeks)	22	12	19	8	30	32	24	48	21	39
Ongoing pain (VAS)	0	0	1	0	0	0	0	1	0	0
Allodynia	No	No	No	No	No	No	No	No	No	No
Motor dysfunction (FDI)	Minor	Minor	Minor	Minor	Minor	Minor	Minor	Minor	Minor	Minor
Taking drugs	No	Yes	No	Yes	Yes	No	No	Yes	No	Yes
School absence	No	No	No	No	No	No	No	No	No	No

rence of symptoms after the epidural catheter was removed.

Finally, three patients who did not respond to systemic medication or to neuraxial analgesia needed the surgical placement of an octopolar paddle spinal cord stimulator to relieve their pain. This last group of patients improved their clinical situation significantly with this treatment, making better physiotherapeutic management possible and reducing and/or abolishing the need for any oral medication (Table 2).

None of our patients reported mild or important secondary effects due to any of the therapies used during the study. Some minor side effects such as transient dizziness, dry mouth, or minor pain after surgery were reported in 30% of cases.

DISCUSSION

Complex regional pain syndrome is usually represented by a complex clinical presentation and[4–6,29,30] a pathophysiology that seems to be multifactorial in nature, characterized by an aberrant host response to tissue damage. Most of the clinical features of this condition are apparent in the confluence of three major pathophysiological pathways: vasomotor dysfunction, aberrant inflammatory mechanisms, and maladaptive neuroplasticity. The clinical heterogeneity of the disorder is indicative of the interindividual variability in the activation of these pathways after tissue injury.[6,31,32]

Several authors have highlighted differences in the pediatric presentation compared to that of adults with CRPS[33] (Table 3). Abnormal intensity and burning pain, in addition to mechanical allodynia, dysesthesia, and paresthesia, are present in nearly 100% of the cases reported in the literature. Signs of autonomous dysfunction such as edema and discoloration are present along with a significant reduction in movement range of the affected limb.[8,9,11,30,34] The symptoms displayed in our patients were in most cases the majority of those described above. The complexity of CRPS shown in this group of children was outstanding as the majority took a long time to be diagnosed and treated appropriately for their condition.

In our experience, the prognosis of the disorder substantially depends on an early diagnosis. Most studies agree with this finding.[8,11,14,15] Low et al.[35] showed that children who get a prompt diagnosis (< 12 weeks) and therefore rapid treatment achieved a quicker and more successful remission of CRPS when compared to those whose diagnosis was delayed (10.6 and 21.5 weeks). In spite of this, Murray et al.[15] could not corroborate the relationship between early onset of treatment and early recovery. Still, we support that early detection and diagnosis, using standards such as the Budapest criteria to ensure this, can attenuate the possibility of permanent suffering, as delayed diagnosis may result in lifelong pain, functional deficiency, and psychological complications.[27,34] Unfortunately, children are often diagnosed in a delayed fashion because CRPS is still a rare and unknown condition in this

Table 3. Adult vs. Pediatric CRPS Characteristics

Characteristic	Adult	Pediatric
Age*	45	12
Gender ratio	Male predominance	Female predominance
Extremity affected	Upper	Lower
Trauma	Mild–severe	Minor–mild
Limb temperature	30% cooler	70% cooler
Edema	40%	75%
Prognosis	Variable, long-term disability	Excellent recovery in most cases
Relapse rate	10%	30%

*Mean age at presentation of the symptoms.

population. Frequently, children will have seen multiple healthcare providers before a formal diagnosis is made, eventually leading to the symptoms described above.[36]

Several characteristics have been identified that differentiate CRPS in children and adults (Table 3)[9,10]; however, these are not only clinical. During childhood, CRPS usually appears in the lower extremities, whereas the upper limb is most commonly affected in adults.[11,27] To further this point, Tan et al. demonstrated in a retrospective study of young patients that the upper limb was affected in only 23.3% of these patients vs. the 72.6% who had an affected lower limb.[33] In our study, the difference was even more notable as all patients presented CRPS in the lower extremities. Another difference identified in the literature between adults and children is that females are more often affected than males (7:1).[27,35] However, we did not find this ratio, as our study had an equal proportion of male to female patients. Finally, another interesting detail found in this study is that children show complete recovery of any lost functionality after correct management, whereas adults retain some disability after the symptoms are resolved.[2,11,37,38]

Most clinicians agree that the best possible prognosis is achieved with an diagnosis and treatment.[8,14,15] Additionally, the medical community agrees that the cornerstone outcome should be the restoration of function. Acknowledged therapies include a combination of pharmacotherapy, physical therapy, and psychotherapy where appropriate.[1,14,16–18] Several studies have shown that early mobilization of the affected limb assisted with cognitive behavioral techniques is the most important part of the management process in childhood.[14,17,37] In our experience, this has remarkable importance, but so does the use of medication as it was shown to be indispensable in pain reduction. This is why we provide multidisciplinary management, including physiotherapy, psychotherapy, and pharmacological treatment as soon as patients arrive at the pain clinic.

Among the drugs used to treat CRPS, NSAIDs, acetaminophen, tramadol, tricyclic antidepressants, and gabapentinoid anticonvulsants are frequently recommended.[16,35] However, despite different combinations and dose escalation of these drugs, some patients do not improve in terms of sensations such as allodynia in severe cases. With the aim of reducing this, we began treatment with a capsaicin 8% patch in those who presented severe allodynia. The capsaicin 8% patch is not currently FDA or EMA approved for use in children. We meticulously explained to the children and their families the mechanism of action of the patch in adults, the risks associated with its use, secondary effects, different therapeutic options, and, more importantly, our aims and hypothesis. After this, the parents of seven children signed the consent form approving the use of the patch. The capsaicin patch was applied in the seven children, providing significant relief for allodynia, hyperalgesia, and dysesthesia in five of them. In three patients, the capsaicin 8% patch together with oral medication and physical and psychological treatment eradicated all symptoms. Here, the application of the patch was the final and decisive step in their treatment. Notably, none of the children treated suffered any side effects. This new and improved topical formulation has emerged as an effective tool to treat chronic refractory pain in adults; however, to our knowledge, it has not yet been documented for use in CRPS during childhood. This is an important tool that should be incorporated as part of a complex analgesic regimen for improving pain management plans in the pediatric CRPS population.

Only those patients who did not improve successfully after being treated with the three-pillar pain management plan described above were candidates for invasive pain therapies. The implications of invasive managements in children who do not respond to conventional treatments are not established, and a positive publication bias must be assumed. Nevertheless, there are numerous reports of treatment success using invasive techniques,[22,23,26,39,40] providing doctors new alternatives when noninvasive options are not enough.

In this clinical case series, six patients (60%) required these techniques after exhausting all other possibilities. Pain and motor incapability continued progressing despite noninvasive treatment for more than 4 weeks at the pain unit. In the operating room and under inhalation anesthesia, we placed a lumbar epidural catheter for bupivacaine infusion, as described in Figure 1. Doctors at the Pain Treatment Unit managed the local anesthetic infusion externally, which was titrated carefully until there was a decrease in pain without side effects. The infusion continued for 2 weeks, after which the catheter was removed and the subject was evaluated. In three of our patients, pain and other symptoms decreased permanently, co-medication was reduced, and physical therapy management helped significantly. On the other hand, in the other three patients, symptoms reappeared after an initial period of improvement.

In the unsuccessful cases, SCS was the next option for treatment. After obtaining consent, we surgically placed

an octopolar paddle spinal cord stimulator. This last group of patients improved their clinical situation significantly with this treatment, making better physiotherapeutic management possible and reducing and/or abolishing any oral medication. In the line of this study, we have found several reports on the use of SCS in children.[26] However, no curative effect of SCS has been reported, not even in adults. The mechanism behind the beneficial effects of SCS is basically unknown, but reviews of the present knowledge and some hypotheses relating to CRPS have started to come to light.[41] From our point of view, SCS is a minimally invasive and reversible treatment method that can be useful in the management of otherwise therapy-resistant CRPS presentations in children.

This case series suggests a multidisciplinary management approach to the treatment of CRPS in children for whom the standard treatment was not successful. Because of the severity and rapid progression of symptoms in CRPS, we consider that early diagnosis of the condition together with comprehensive and individualized multidisciplinary treatment offers children with CRPS the best opportunity for complete recovery. Within this management plan, novel drugs should be included, such as the capsaicin 8% patch, in addition to invasive techniques for patients who otherwise do not respond to noninvasive therapies. Thus, a more aggressive approach needs to be attempted. Further research into CRPS in children is needed, and new treatment guidelines are required for those children who do not respond to established management modalities.

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Comprehensive Review

Invasive Management for Pediatric Complex Regional Pain Syndrome: Literature Review of Evidence

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Background: Complex regional pain syndrome (CRPS) is a multifactorial condition with complex pathogenesis characterized by spontaneous or stimulus-induced pain that is disproportionate to the inciting event. It is also commonly accompanied by a myriad of autonomic and motor disturbances in highly variable combinations. This condition has been underreported in children until recently. Consequently, the management of CRPS in the pediatric population presents an even greater challenge than in adults, partly because there is a lack of clinical data concerning the efficacy of the diverse treatment methods available, and partly because successful treatment of CRPS involves a multidisciplinary approach. There is a variety of invasive methods to the treatment of CRPS, but scarce pediatric-focused trials have been published to date.

Objective: To examine and analyze the data currently existing for the invasive management of CRPS in children. It further suggests a management algorithm based in the evidence reviewed and our team experience.

Study Design: A comprehensive review of invasive management for pediatric CRPS.

Setting: Academic hospital in Spain.

Methods: A comprehensive review of all the evidence published to date was conducted. Four databases (PubMed, Medline, Web of Science, Embase, and Cochrane databases) were searched for articles published from 1980 to 2014. The eligibility criteria were any paper published in English or Spanish where a non-conventional approach was used to manage pediatric CRPS. Two independent reviewers extracted the data.

Results: Many case series have reported the use of interventional management with positive results; however, there is not a single randomized control trial to date comparing the conservative and the invasive management in children. The largest series of pediatric cases showed that between 29% to 35% of children with CRPS needed interventional measures to manage this condition successfully. Sympathetic blocks and spinal drug infusion emerge as the most reported techniques; the spinal infusion of drugs together with the spinal cord stimulation being the most successfully employed. Based upon the available evidence with regard to effect and complications, we recommend an algorithm for the management of pediatric CRPS.

Limitations: The limitations of this study include the paucity of literature, lack of randomized trials, and lack of quality evidence.

Conclusions: Invasive techniques have been used to treat CRPS over the last few decades; however, the evidence for their use is still very weak. Invasive management should be contemplated only when high-standard conservative management has failed to work.

Key words: Pediatric pain, complex regional pain syndrome, CRPS, invasive treatment, pain management, multidisciplinary management, neurostimulation

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Complex regional pain syndrome (CRPS) is a term defined by the International Association for the Study of Pain (IASP) to describe disorders primarily characterized by spontaneous or stimulus-induced pain that is disproportionate to the inciting event. CRPS has been suggested to be a multifactorial disorder that is related to an aberrant host response to tissue damage (1,2). The disease often includes a wide variety of autonomic and motor disturbances in highly variable combinations (3,4). The symptoms can be categorized into 2 groups: positive noxious symptoms, such as hyperalgesia and allodynia, and negative symptoms of sensory loss (1,2,5). Usually, patients with CRPS present following moderate or insignificant tissue damage. In the acute phase, the patient can exhibit an extremely painful, red, warm, and swollen injured limb. Other potential accompanying features are changes in sweating, hair and nail growth, allodynia and hyperalgesia, and muscle weakness. As the disorder continues, pain spreads, voluntary motor control is reduced in most patients, and negative sensory signs, namely hypoalgesia and hypoesthesia, become more apparent (1,6,7).

CRPS has been extensively studied in adults, while studies in children are scarce (1,8,9). For a long time it was doubtful that this condition even existed in children, nonetheless within the last few years numerous articles have reported CRPS at young ages (Table 1). However, due to the lack of understanding regarding its precise pathophysiology, reliable diagnostic tests are not available. CRPS diagnosis entirely depends on observable signs and reported symptoms, which have been put together into various diagnostic criteria sets for adults (4,10,11). Unfortunately these diagnostic criteria do not often agree, raising a high degree of uncertainty into a CRPS diagnosis. To date specificity and sensitivity of the standard diagnostic criteria sets have not been evaluated for pediatric patients.

As well as posing a significant diagnostic challenge, the timely diagnosis of CRPS can substantially influence the prognosis (12-15). Additionally, prompt and accurate management is key, where the cornerstone is to restore function of the affected limb. Recognized therapies include a combination of pharmacotherapy, physical therapies, and psychotherapy where appropriate (14,16-19). Only patients who fail to progress with physical therapy may require additional or more invasive pain therapy, such as spinal cord stimulation (SCS), intraspinal analgesic infusion, or sympathetic blocks (20-23). Neurostimulation therapy and spinal cord drug infusion have been available since the 1970s and have grown in acceptance in recent years for the treatment of pain disorders of diverse etiology (21,24). Today, CRPS in adults is the second largest indication for the use of SCS in the United States, reaching success rates of up to 70% in pain reduction in CRPS sufferers treated with SCS when properly selected (25,26). However, the significance of invasive procedures during childhood and adolescence for the treatment of CRPS patients who do not respond to conventional treatments or medications continues to be unestablished (27). Several reports in the literature demonstrate success with these procedures, providing physicians (or clinicians) with more alternatives after conventional options fail (Table 3).

The focus of this article is to review the evidence for invasive pain procedures along with presenting a management algorithm for pediatric CRPS, including invasive procedures for patients who do not respond to the conventional first-line treatment.

Table 1. *Invasive interventions for complex regional pain syndrome.*

Intervention	n studies (%) N = 31	n patients	1980 – 2000	2000 – 2015	Reference
Sympathetic blockade (singular or continuous)	15 (48%)	123	7	8	(11, 38, 40, 58, 65-75)
Spinal drug infusion or epidural catheter	11 (35.5%)	25	0	11 (100%)	(29, 33, 38, 51, 58, 69, 71, 74, 76-78)
Regional anesthesia	10 (32%)	36	1	8 (91%)	(11, 29, 32, 38, 43, 69, 74, 79, 80)
Intravenous lidocaine	7 (22.4%)	28	4	3	(40, 58, 66-68, 81, 82)
Spinal Cord Stimulation	3 (9.6%)	11	0	3 (100%)	(28, 33, 76)
Surgery	3 (9.6%)	5	3	0 (0%)	(81, 83, 84)
Sympathectomy	2 (6.4%)	28	2	0 (0%)	(44, 81)
	31 (100%)	171			

Table 2. *Adult vs. pediatric CRPS characteristics.*

Characteristic	Adult 1	Pediatric 2
Age*	45	12.8
Gender ratio	Male predominance	Female predominance (85%)
Extremity affected	Upper	Lower (80%)
Trauma	Mild- Severe	Minor- Mild
Limb temperature	30% cooler	70% cooler
Edema	40%	75%
Prognosis	Variable, long term disability	Excellent recovery in most cases
Relapse rate	10%	30%

* mean age at presentation of the symptoms. [1]Data extracted from CRPS adult literature. [2] Description of patients comprised in this review.

Table 3. *Relevant publications, selection by the authors.*

Study	Year	Interven-tion	n	Outcome measur.[1]	Length[2]	Previous medication[3]	Adverse effects	Improvement (% patients)	Comments
Rodriguez et al (33)	2015	LA Spinal inf. SCS	10 (6)*	Yes	52 w	Opioids (67%) NSAIDs (83%) Anticonvul (100%) Antidepress (67%) Capsaicin (100%)	No	100%	This study showed successful results after applying a multimodal and progressive approach including invasive measures as well as physical management and novel medication as the capsaicin 8% patch.
Olsson et al (28)	2012	SCS	7	Yes	52/250 w	Opioids NSAIDs Anticonvul Antidepress Ketamine (14%) Epidural L.A (28%)	Yes, Local infection	Full recovery (72%) Minor symptoms or recurrences (28%)	Olsson´s study comprised 7 girls, presenting with severe, incapacitating and therapy-resistant CRPS-I, who were subjected to SCS. Good technique description but poor methodology.
Meier et al (42)	2009	Cont Lumbar sympath block Lidocaine iv	23	Yes	-	"6-week trial of aggressive physical, bio-behavioral, and pharmacological therapies"	Minor	LSB: Complete (29%). Adequate (41%) Minimal (32%) Lido iv: Minimal (84%) Adequate (16%)	The purpose of this study is to compare the efficacy of lidocaine administered by lumbar sympathetic to IV route. Excellent methodology and clear results. No follow-up period.
Kachko et al (38)	2008	Epid cath (1) Stellate gang block (1) Regional anesth. (2)	14 (4)*	Poorly	8 w	NSAIDs Anticonvulsive Antidepressant	-	Full (78%) Partial (15%) Recurrence (29%)	Retrospective study that aimed to assess the efficiency of the multimodal management of CRPS. Limited but illustrative of the actual clinical set up of many pain treatment units.
Stanton et al (11)	1993	Sympath block Regional anesth	36 (x)*	Poor	-	NSAIDs Anticonvulsive Antidepressant Opioids	-	Moderate or poor	Review of the experience at this center. They aimed to present diagnostic criteria for pediatric CRPS. Management and outcomes poorly described.
Wilder et al (75)	1992	Sympath block	70 (37)	Yes	20 w	NSAIDs Antidepressant	-	Full (71%) Moderate (13%)	Wilder retrospective study reported his experience with a multimodal treatment, using more than 50% invasive techniques.

(1) Outcome measure carefully described. (2) Length of the follow-up – weeks. (3) Medication prior invasive treatment. * Number of patients treated with invasive measures within the total of patients.

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Literature Selection

A literature search identified studies relevant to invasive treatments for CRPS in children. Databases used included PubMed, Medline, Web of Science, Embase, and Cochrane. Because of the small volume of literature on the pediatric population, database-specific controlled vocabulary (subject headings or index terms) was not used, and keyword searching produced a comprehensive and manageable yield. The following search strategy was used: ((complex regional pain syndrome) OR (CRPS) OR (reflex dystrophy) OR (algodystrophy) OR (causalgia) OR (Sudeck's atrophy) AND (sympathetic OR neurovascular)) OR ((amplified OR complex OR chronic) AND (neuralgia OR pain) AND musculoskeletal)) AND (therapy OR therapies OR therapeutic)) OR (transcranial AND magnetic AND stimulation) (OR spinal cord stimulation OR neurostimulation OR spinal drug infusion OR intra-spinal therapy OR epidural infusion OR epidural catheters OR sympathetic block OR sympathetic blockade OR peripheral blocks OR surgery) AND (child OR adolescent OR pediatric). Initial search results were limited to English and Spanish language articles. The references in the selected articles were used to identify additional relevant sources. In addition, the authors identified a limited number of articles or chapters from personal readings.

Thirty-one studies met the criteria to be included in this review (Table 1). Their full texts were analyzed for retrieving information such as the invasive treatment –used – including prior and concurrent conservative interventions, outcomes measured, type of study, patient characteristics, quality of the study, design, and methodology.

Review of the Evidence

Conservative Management

Although reviewing CRPS non-invasive therapy is not the goal of this article, we have considered it appropriate to briefly describe the most accepted model of management for this condition. CRPS in children and adolescents seems to respond favorably to conservative multimodal inpatient therapy (34,35). In the largest pediatric trial reported to date, 92% of children and adolescents were free of symptoms after an intensive physical therapy program (36). Other smaller series identified in the literature have presented recovery rates of 70% as well after applying conservative management (25,37,38), however recovery or resolution is not always well-defined.

Nonetheless the long-term prognosis is unclear and between 28% and 48% of patients with pediatric CRPS experience a relapse (16,25,36,37,39). Consolidation of the evidence suggests that conservative treatment of pediatric CRPS should form the basis of first-line treatment. Being the medication, the psychological and the physical therapies are clearly the core of the initial treatment. However, further interventions are needed when the condition does not resolve or a relapse occurs.

Invasive Pain Therapy

The relevance of invasive therapies in children who otherwise do not respond to conservative management or medications after a few weeks of treatment has not been established in pediatric patients (27,28,33,40). There is not a single randomized control trial to date comparing the conservative and the invasive management of this particular group of patients. The largest series of pediatric cases showed that between 29% to 35% of children with CRPS needed interventional measures to manage this condition successfully (14,38,41).

Within this review we have identified 31 publications published between 1980 and 2015. Most studies were case series and case reports (n = 28), including a total of 108 patients. One randomized control trial of 23 patients and 2 controlled studies of 40 patients in total complete the collection of studies of this review (Table 1). The entire collection of publications contained data of 171 patients. The characteristics of the population who received invasive procedures correlates with the characteristics of the children shown in other publications affected by this syndrome who do not receive this sort of treatment (Table 2) (23,39). Spontaneous pain and functional disability were the 2 outcomes measured with more assiduity. The overall improvement for spontaneous pain was documented in 79% of cases; 16% of patients showed no change. Functional disability was reported in 25 publications, 24 of them showed improvement after treatment.

This study reveals that the most used procedure was the sympathetic blockade (Table 1). Singular or continuous sympathetic blocks were used in 15 studies, 123 patients. Within this group of studies we found the only randomized control trial (42) and 2 controlled studies (43,44). Numerous types of blocks are included in this group, for example: the sympathetic blocks of the ganglion stellatum for CRPS in the arm, the block of the lumbar truncus sympathicus for CRPS in the leg, or the thoracic block of the Kuntz's nerve. Local anesthetic blockade of the sympathetic chain has been widely

used to treat CRPS in adults, however the empirical data is confusing (45,46). A systematic review revealed the paucity of published evidence to support the use of local anesthetic sympathetic blockade as the "gold standard" treatment for CRPS (32,47,48). Likewise, we can conclude that its efficacy has not been proven for the treatment of CRPS in adults. The data in children is far scarcer and uncertain, that is why this treatment has been relegated to a more tentative choice in pediatric CRPS. Additionally, most of the publications analyzed revealed that multiple invasive procedures were needed during the period of treatment with this technique, increasing the risks of side effects (11,38).

The spinal drug infusion of local anesthetics was used in 11 studies, all of them in the last 15 years. Spinal drug infusion through epidural catheter has been largely used in this group of patients when the physiotherapy program needs to be supported or when the symptoms do not decrease with conservative management. Epidural drug infusion with local analgesics is a viable alternative when conventional treatments do not achieve acceptable results, it also has the advantage of supplementation with opiates to the local anesthetics to offer better analgesia. The complications and risks of this technique (e.g., respiratory depression, motor block, sympathetic block resulting in hypotension, and urinary retention) can be avoided by careful titration of the infused medications and adequate patient and family education. To date there is no randomized trial for spinal drug infusion in CRPS, however there are numerous reports supporting this technique. Of 37 adult CRPS patients treated with continuous epidural infusion of bupivacaine and fentanyl, nearly 90% had a reduction in their symptoms when treated within 12 months after onset. However, the success rate diminished considerably when treatment was started more than a year after onset (49). In the pediatric literature, reports are fewer yet analogous to those found in adults which would suggest a favorable outcome (33,38,50,51). Some authors highlight the importance of avoiding delay for treating CRPS invasively (33,50). Therefore, we conclude that early treatment with continuous epidural anesthesia may be promising when initial non-invasive management is ineffective.

SCS has demonstrated efficacy in CRPS type 1 in adults (30,52,53). In SCS in adults, as in pediatrics, an electrode is placed in the epidural space on the dorsal aspect of the spinal cord at the level of the nerve roots innervating the painful area. Electrical current from the electrode brings about paresthesia, a sensation that suppresses the pain. This technique has become more popular during the last decade for the management of CRPS in adults, obtaining successful results in most cases (21,53-55). In the pediatric population it has been suggested as a possible option when the patient is resistant to all conventional treatments (30,55-57), but only a few examples of successfully treated CRPS in children have been presented to date, 3 case series with 11 people in total (16,28,33). Therefore, to the best of our knowledge SCS can be a useful and promising treatment for CRPS in pediatric patients who do not respond to conventional treatment. Nevertheless, due to the small and non-controlled design of these case series, further studies are needed to verify that SCS can be recommended for its use in this group of patients.

There are others invasive techniques that have been considered when conventional therapy has failed in pediatric CRPS. Regional anesthesia has been tried in 36 patients during the last years, mostly during the last 10 years, however the results do not appear to be as good as with some of the techniques mentioned previously. Similarly, intravenous regional blocks with lidocaine show unsatisfactory or unclear results in general: the decrease in spontaneous pain and functional disability improvement less than with any other procedure, 55% and 50%, respectively.

Discussion

CRPS is characterized by complex clinical presentations and a pathophysiology that seems to be multifactorial in nature, characterized by an aberrant host response to tissue damage (1,5,58). Most of the clinical features of this condition can be explained by the confluence of 3 major pathophysiological pathways: vasomotor dysfunction, aberrant inflammatory mechanisms, and maladaptive neuroplasticity. The clinical heterogeneity of the disorder is indicative of the inter-individual variability in the activation of these pathways after tissue injury (1,59,60).

The recommendations of the special interest group in Neuropathic Pain (NeupSIG) of the IASP for the pharmacological management of neuropathic pain (NP) only considered treatments with at least 2 high-quality randomized clinical trials (61). Nonetheless, there is limited evidence evaluating interventional treatments for NP, and many interventions used in clinical practice to manage NP in refractory patients are supported by weak, if any, evidence (62). This evidence is even more fragile when talking about the management of CRPS,

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and completely exiguous when referring to the management of pediatric CRPS.

Nonetheless, the scientific consensus is that the cornerstone of CRPS management should be the restoration of function. Acknowledged therapies include a combination of pharmacotherapy, physical therapies, and psychotherapy where appropriate (14,17-19). Several studies highlight that early mobilization of the affected limb assisted with cognitive behavioral techniques is the most important part of the management process in children (14,16). In our experience this is highly important but so is the use of medication and the early diagnosis of the disorder, which substantially influence the prognosis of the condition (12-15). Low et al (37) showed that children who received a prompt diagnosis (less than 12 weeks), and therefore were offered treatment more rapidly, achieved a quicker and more successful remission of CRPS when compared to those whose diagnosis was delayed (10.6 and 21.5 weeks).

Unfortunately a significant percentage of children who suffered CRPS do not respond to conservative treatments. Only those patients who do not improve successfully after being treated with a complete pain management plan during a reasonable time are candidates for invasive pain therapies (33). Unfortunately the evidence supporting the use of these procedures is weak. This review shows that the methodological quality of the existing data is low as most of the publications found are case series or case reports representing level IV evidence. On top of that, a very low percentage of publications used the established diagnostic criteria for CRPS from the IASP. Additional negative aspects of this group of publications are that validated outcome tools were not used in most cases and that the follow-up periods were usually not reported or rather too short.

Within the invasive techniques described in these publications, we must highlight the continuous epidural infusion and the SCS. They seem to have an important effectiveness and to be minimally invasive and reversible, besides in adults, they have been shown to be very effective for certain forms of NP (49,62,63). Olsson et al (28) concluded that SCS was successful for treating CRPS in all their pediatric patients; however, this conclusion is questionable from our point of view as in one of the patients the symptoms ceased after the patient had not responded well to any stimulus of SCS and another patient in the same series developed an infection which seriously compromised the treatment. Rodriguez et al (33) had a great experience with SCS, abolishing the symptoms in 3 children with a well-defined history of uncontrollable CRPS. This study, together with the positive experience of Wilder (16), encourages the need for a better understanding and use of SCS in CRPS. Likewise, the use of an epidural catheter for the infusion of local anesthetics has been implemented in the last years. The majority of publications agreed that the treatment diminishes the pain and improves functionality of the limb affected. Regrettably, very few of these publications described the process (the space where the catheter was implanted, the concentration, the dose, etc.), the outcome, or the side effects, if any.

Side effects were infrequently reported. Infections only occurred in 2 patients and minor side effects were reported only in 10 studies. Sixteen of 83 reported cases experienced a relapse. From our perspective, based on our experience and the literature behind these procedures, we believe that the side effects in this collection are underreported.

Recommendation

Based upon the available evidence with regard to effect and complications, we recommend the following algorithm for the management of pediatric CRPS (Fig. 1).

A crucial first step for the management of this condition appropriately consists of making an accurate and early diagnosis. We strongly encourage basing the diagnosis in the CRPS criteria from the IASP (10), despite that this set of criteria has been made for adults. The real goal of the physician must be the restoration of the normal function of the affected limb, using every possible management tool to achieve this. Initially, physical therapy, psychological support, and adequate pharmacological treatment should be used together, complementing one another and aiming to make the condition resolve within a few weeks. Pharmacological measures are prescribed on a symptom-oriented basis. However, new approaches should be adopted when fitting within a mechanism-based management (8). Analgesics, anti-inflammatory therapy, and antidepressant and antiepileptic drugs have been used to date. However, new topical drugs such as the high-concentration capsaicin patch have been tried within the past few years with excellent results (33).

Based on our experience (33), the heterogeneity found in the literature regarding the duration of the conservative management for treating CRPS together with the lack of knowledge of its precise pathophysiology, we recommend that after a reasonable time of 4 to 5 weeks under intensive multimodal therapy with-

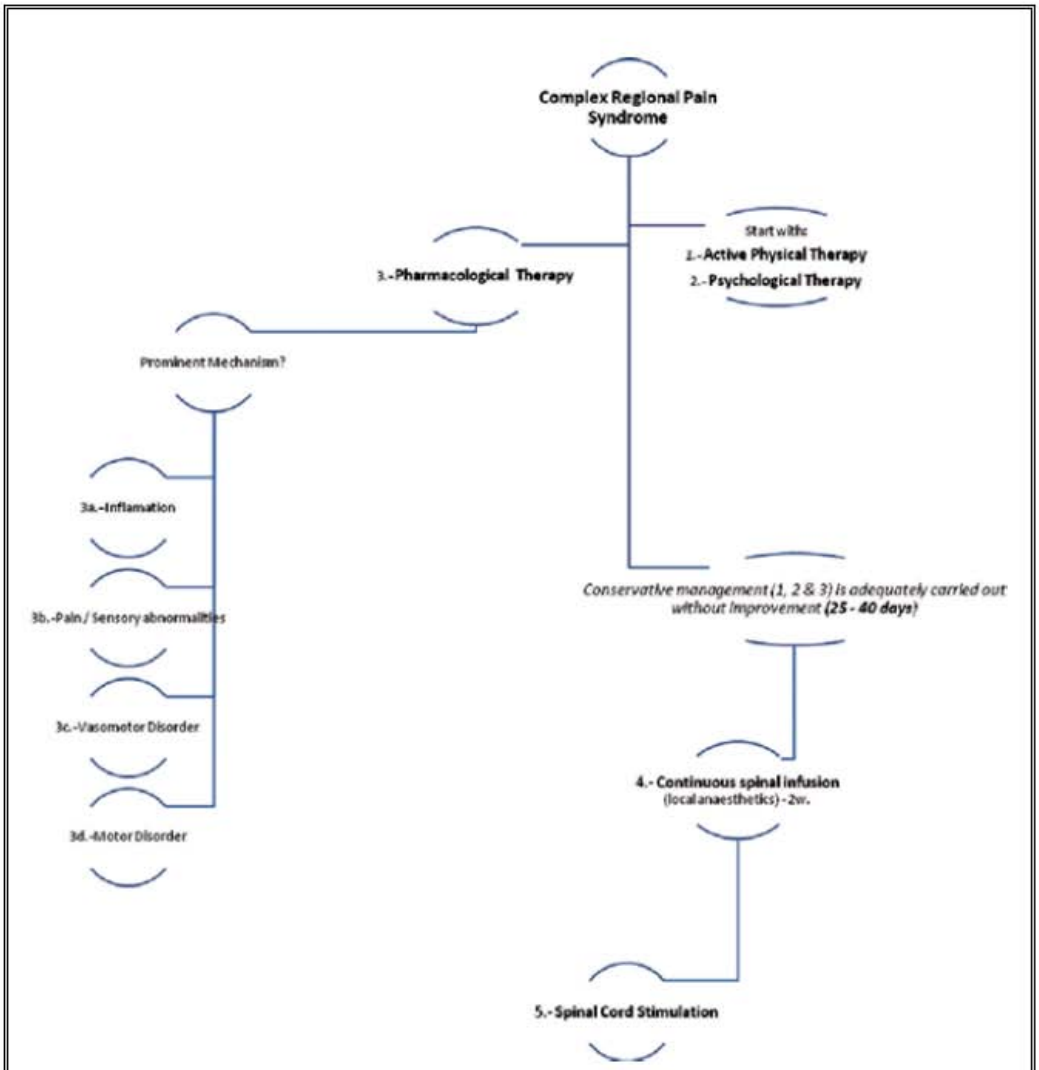

Fig. 1. *Clinical algorithm for the management of pediatric CRPS. The first crucial step is the correct and early diagnosis. Immediately after, a multimodal approach must be taken. Active physical therapy, psychological support, and the correct pharmacological approach must be planned. Initially a sign and symptom oriented pharmacological treatment is engaged. Patients who do not respond succesfully to the conservative management carried out during a period of 25 to 40 days can be treated with invasive techniques. First LA continuous epidural infusion for 2 weeks and then SCS can be recommended after multidisciplinary evaluation.*

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out successful results, more invasive options should be considered. Before failure of conservative management is taken as a reason to contemplate invasive measures as the following step, only high-quality conservative treatment should be implemented. Therefore, knowledge concerning such treatment needs to be increased. Patients with CRPS with severe pain, allodynia, or with a measurable skin temperature difference compared to the non-affected limb that do not respond to the multi-modal conservative management should be put forward for therapies such as spinal infusion of drugs, sympathetic blockades, or SCS. In our opinion, after reviewing the literature on the topic, the initial option for children who do not respond successfully to conservative management is the continuous epidural infusion of local anesthetics (33). This technique provides analgesia and sympathetic blockade throughout the time the catheter is attached. This is usually enough to control the condition and prevent the reappearance of symptoms (33). We recommend its use for 10 to 14 days. Having the catheter in for more time could increase the risk of infection (64) or further side effects secondary to the drug infusion or the catheter itself. However, this technique can fail or the symptoms can re-emerge after the catheter is removed. In this case, SCS can be recommended after multidisciplinary evaluation and a successful trial stimulation. SCS is a minimally invasive and reversible technique that facilitates physical therapy and helps decrease medication (28).

Conclusion

This article proposes a multidisciplinary management approach to the treatment of CRPS in children for whom the standard treatment has not been successful. Because of the severity and rapid progression of the symptoms in CRPS, we consider that an early diagnosis of the condition together with comprehensive and individualized multidisciplinary treatment offers children with CRPS the best opportunity for a complete recovery. Within this approach we encourage clinicians to consider invasive procedures as a reliable option of treatment. Unfortunately the type of technique that should be applied when high quality multimodal conservative treatment fails cannot yet be based on empirical data. Therefore, since there is significant limitations of the evidence, interventional treatments for the management of CRPS in children should ideally be offered in clinical and research settings with experience and ability to understand and report the outcomes. This will make it possible to substantially improve the evidence on which forthcoming recommendations are established.

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Other publications of the author.

Date: 23-26 May. 2013
Congress: 4th international congress of neuropathic pain
Poster: - Comparison of responses to the painDetect questionnaire and QST in patients with neuropathic pain (1st author)
- Does the pain rating correlate with distinct QST parameters? (2nd author)
Creditos: 22 CME

Date: 15-17 Octubre 2013
Congress: Reunión Andaluza AAD 2013
Poster: Comparison of responses to the painDetect questionnaire and QST in patients with neuropathic pain Español. (1st author)

Date: 3-6 Sept 2014
Congress: 33rd Annual ESRA international
Poster: -percutaneous micro-compression of the gasserian ganglion for trigeminal neuralgia (2nd author)
- Are we underdosing our analgesic prescriptions? a prospective observational study on 400 patients (2nd author)
- Unusual presentation of post-lumbar puncture headache requiring epidural blood patch (3rd author)

Date:18-20 Sept 2014.
Congress: The anual congress of the European Society of Paediatric Anaestesiology (ESPA) 2014, Prague, Czech Republic
Poster: interventional treatment of complex regional pain syndrome in children

Date: 26th -30th Octubre 2014
Congress: Montescano EFIC Pain School. Neurological Diagnosis in Chronic Pain

Date 27-28-29 Noviembre 2014
Congress: 59 Reunion AAEAR
Poster: Uso del parche de Capasaicina al 8%en tratamiento del SDRC (1st autor)
- Irrigacion de la cavidad torácica: una inusual causa de SCASEST. (2nd autor)

Date 13-17 May 2015. Nice, France
Congress: NeupSIG Reunión Ineternacional de Dolor Neuropático.
Poster: CRPS in children (1st autor)-

Date : 15 al 17 de Octubre de 2015, Santander.
Congress: XXXIII Congress de la Sociedad Española de Anestesiología, Reanimación y Terapéutica del Dolor - SEDAR
Poster:
- (1st autor) - Uso del test de cuantificaci. n sensorial (qst) en el tratamiento y seguimiento de pacientes con dolor neuropático.
- (1st autor)- Manejo y consideraciones anestésicas de la enfermedad de menkes. a propósito de un caso.
- (1er autor) Eficacia de diferentes estrategias de reversión en la disminución de incidencia del bloqueo neuromuscular residual en URPA: estudio analítico prospectivo de cohortes.
- (2nd autor) Determinación de incidencia de bloqueo neuromuscular residual tras cirugía digestiva: estudio observacional descriptivo
- (3er autor) El género como factor de riesgo de bloqueo neuromuscular residual en urpa: estudio analítico prospectivo de cohortes

Date : Noviembre 2015. Córdoba
Congress: 60º Reunión AAEAR
Poster:
- (1st autor) Dolor Neuropático y Enfermedad de Fabry. A propósito de un caso
- (1st autor) Bloqueo auriculo ventricular congénito (BAV), manejo anestésico.
- (1st autor) Manejo anestésico obstétrico de la paciente con Neurofibromatosis Segmentaria

Date: Noviembre 2015
Congress: Reunión Andaluza Residentes de Traumatología (SATO) 2015
Poster/ presentación: (2nd autor) - Dolor neuropático en artropatía degenerativa

Date : Mayo 2015. Sevilla
Congress: XIII Reunión Iberoamericana del Dolor
Poster: (1st autor) Serie de 10 casos de SDRC en niños. Nuevas posibilidades terapéuticas.

Date : Mayo 2016. Pamplona.
Congress: XIV Reunión Iberoamericana del Dolor
Poster: (1st autor) Programa de educación en neurociencias y dolor para pacientes con dolor neuropático crónico: Estudio piloto. (electo como publicación para la revista de la sociedad española de dolor)

Date : Noviembre 2016. Almería
Congress: 61º Reunión AAEAR. Asociación Andaluza Extremeña de Anestesiología y Reanimacion.
Poster: (1st autor) Factores de riesgo de insuficiencia renal aguda en pacientes sometidos a cirugía supratentorial.

Date: 4-6 Mayo 2017. Alicante
Congress: XXXIII Congress de la Sociedad Española de Anestesiología, Reanimación y Terapéutica del Dolor - SEDAR
Oral presentation: Papel de la ecografía en la colocación del tubo endotraqueal de doble luz.
Poster:
-(1st autor) Identificación de factores de riesgo para la insuficiencia renal aguda postoperatoria tras el uso de manitol en neurocirugía.
- (2nd autor) Ventilación de protección pulmonar en cirugía robótica da vinci